THE PRINCIPLES OF MICROSCOPY

WRIGHT

Battersea Borough Council

PUBLIC LIBRARIES.

This book belongs to the Reference Department of the Central Library, Lavender Hill, and should be returned to the counter when done with.

Under no circumstances must it be taken out of the room.

5,000—231—1/19.

D2388

LONDON BOROUGH OF LEWISHAM
LIBRARY SERVICE

Author

Title

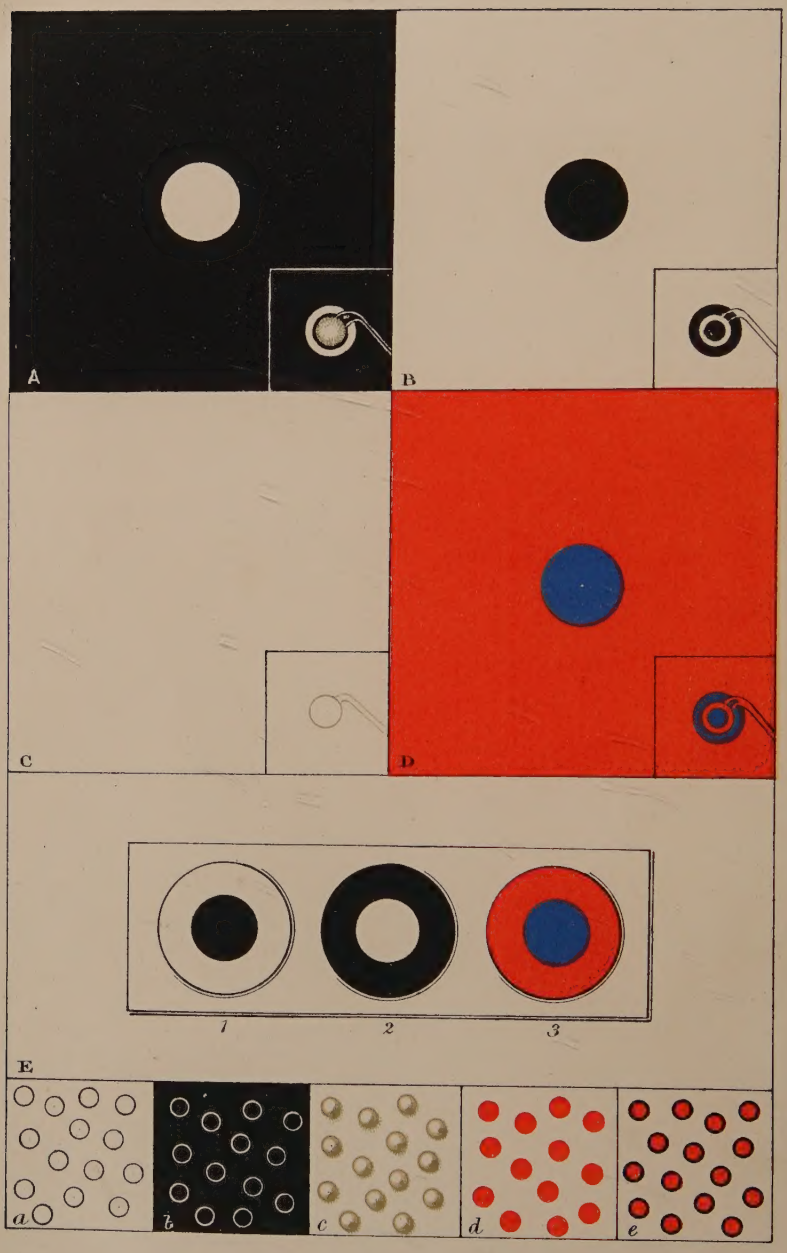
BOOK STORE

Books or discs must be returned on or before the last date stamped on label or on card in book pocket. Books or discs can be renewed by telephone, letter or personal call unless required by another reader. After library hours use the Ansafone Service (01-698 7347). For hours of opening and charges see notices at the above branch, but note that all lending departments close at 1 pm on Wednesday and all libraries are closed on Sundays, Good Friday, Christmas Day, Bank Holidays

METROPOLITAN
SPECIAL COLLECTION

PRINCIPLES OF MICROSCOPY

BATTERSEA
PUBLIC
LIBRARIES



C.F.KELL & SON WESTMINSTER.

Principles of Microscopy

BEING A

Handbook to the Microscope

By

Sir A. E. WRIGHT, M.D. (Dublin)

F.R.S., Hon. D.Sc. (Dublin), Hon. F.R.C.S.I.

Sometime Professor of Pathology, Army Medical School, Netley;
Director in Medical Charge of the Department for Therapeutic Inoculation,
and Pathologist, St. Mary's Hospital, London, W.
Author of "A Short Treatise on Anti-typhoid Inoculation," etc.

LONDON
ARCHIBALD CONSTABLE & CO., LTD.
16 JAMES STREET, HAYMARKET, S.W.
1906

L00125534 12
R 578·4 CATEGORY msc
BOOKSELLER INVOICE DATE
ACC. NUMBER D23988

Reference Library
No 9680
WITHDRAWN.

BUTLER & TANNER,
THE SELWOOD PRINTING WORKS,
FROME, AND LONDON.

BATTERSEA PUBLIC LIBRARIES

PREFACE

EVERY one who has to use the microscope must decide for himself the question as to whether he will do so in accordance with a system of rule of thumb, or whether he will seek to supersede this by a system of reasoned action based upon a study of his instrument and a consideration of the scientific principles of microscopical technique.

The present text-book has no message to those who are content to follow a system of rule of thumb, and to eke this out by blind trial and error.

It addresses itself to those who are dissatisfied with the results thus obtained, and who desire to master the scientific principles of microscopy, even at the price of some intellectual effort.

The general scope of this treatise is briefly as follows—

I have taken as my theme the technique of the microscope; excluding from my purview such technique as has relation only to special branches of microscopic study, or to the employment of the supplementary apparatus which is used in association with the microscope for the purpose of counting, measuring, analysing, or photographing microscopic objects.

In correspondence with what I conceive to be a natural, and a fundamentally important, subdivision of my subject matter, I have divided my treatise into two parts.

In Part I. I treat of the development of the *object or stage-picture*, i.e. of the development upon the stage of the microscope of that pattern of radiant points which constitutes the original of the magnified image formed upon the retina by the microscope.

In Part II. I treat of the development of the magnified *microscopic image*, and of the instrumental adjustments which minister to the satisfactory development of that image.

To every beginner I would have it pointed out that the principles which are set forth in Part I. constitute absolutely the alphabet of microscopy. I would have such beginner appreciate that the magnified image which is formed upon the retina of the observer is rightly considered the joint creation of the optician and the microscopist. I would have him learn that it is for the microscopist to develop upon the stage of the microscope the pattern of radiant points (spoken of above as the *stage-picture*); and for the optician to take in charge the beams which proceed from that stage-picture, and to form out of these the magnified "*microscopic image.*" I would further have it brought home to every beginner that if he fails to develop upon the stage of the microscope a stage-picture, no effort on the part of the optician will conjure up an image; while given a satisfactory stage-picture, even the poorest of microscopes will furnish something to the eye.

Upon the more advanced microscopic worker I would have it impressed that, while it holds true generally that the stage-picture is the concern of the microscopist and the microscopic image the concern of the optician, this statement does not embrace the whole truth. The microscopist has also a responsibility in connexion with the development of the microscopic image, in particular in the case where the high powers of the microscope are employed. Here the optician must perforce count upon the intelligent co-operation of the microscopist.

The general subject matter of my work enunciated, I would desire to explain also certain features in connexion with its exposition.

If the reader demurs to being called upon to begin both in Part I. and Part II. with subject matters which are not directly relevant to the microscope, I would explain that this is done with a view to avoiding the treatment of these questions in a parenthetical manner. The reader will appreciate that the art of exposition is the art of avoiding parentheses.

If, again, the reader sees ground of complaint in the fact that he is required at every moment to put down the book and undertake an experiment, I would submit that no proposition is adequately

apprehended until it has been invested in the apposite mental image. The illustrations and experiments in this book, and the diffraction grating which is supplied in the pocket of the cover, are one and all provided to enable the reader to attain to an adequate apprehension of the statements in the text.

Lastly, if the reader feels that he has a grievance in the fact that a number of new coined terms are employed in this treatise, I would put it to him that a complete vocabulary of technical terms is as essential to a satisfactory exposition as a proper ordering of the subject matter, and the provision of illustrative experiments.

It is in obedience to this consideration that I have, wherever the vocabulary of optics seemed to me to be deficient, coined, or adopted into currency, such new technical terms as seemed to me to be required.

To the objector who urges that the existing vocabulary already adequately subserves the exchange of ideas among those who are conversant with optics, and that the coinage of new words could quite well be avoided by occasional circumlocution and by resort to mathematical formulae, I would submit the following for consideration.

We have here, not the case of an interchange of ideas between physicists conversant with their subject and capable of threading their way through the pitfalls provided by an inadequate and often ambiguous vocabulary, but the case of an author endeavouring to give a clear exposition to a reader who must be assumed to be approaching the subject *de novo*.

Circumlocution and mathematical formulae might, no doubt, be resorted to, but circumlocution is everywhere a temporary, and at all times a difficult, expedient; and the use of mathematical signs as a substitute for speech can be defended only in the case of the inarticulate classes of the learned.

In bringing my task—a task which has occupied many years—to a conclusion, I desire to make it clear that I owe to my friend, Mr. J. W. Gordon, a debt greater than I can express for manifold intellectual help and suggestion. I am rather understating than

overstating my indebtedness to him when I say that if it had not been for the assistance which I have derived from his published papers, from his personal collaboration in experiment, and from his criticism, this book would never have taken shape.

To Miss Gertrude Woodward I have to express my grateful acknowledgments for the skill and patience which she devoted to the preparation of the illustrations.

I am indebted also to my friends, Mr. J. W. Gordon, Mr. Maynard Smith, F.R.C.S., and Lieut.-Col. W. B. Leishman, R.A.M.C., for help in connexion with the illustrations, and to Messrs. Carl Zeiss for the blocks of Figs. 2, 3 and 4 on Plate XII.

Lastly, I would ask my publishers—Messrs. Constable & Co.—to accept my very sincere thanks for the generous way in which they have fallen in with my many onerous suggestions in connexion with the production of this work.

St. Mary's Hospital,
London, W.

LIST OF CONTENTS

PART I

THE OBJECT-PICTURE AND ITS DEVELOPMENT

CHAPTER I

PAGE

GENERAL CONSIDERATIONS WITH REGARD TO THE OBJECT-PICTURE . 3

Definition of the object-picture—Classification of pictures as outline pictures, colour pictures, outline and colour pictures, and pictures in relief respectively—Preliminary consideration of the respective advantages and disadvantages of the different varieties of pictures enumerated above—Advantages and disadvantages of the different varieties of pictures from the point of view of the disclosure of objects which (by reason of their minuteness or distance) subtend only a small angle at the retina—Advantages and disadvantages of the different types of pictures from the point of view of the disclosure of the configuration of the object in plan and relief—Advantages and disadvantages of the different types of pictures from the point of view of the disclosure of the internal structure of the object—Advantages and disadvantages of the different types of pictures from the point of view of their greater or less liability to be vitiated by fallacy.

CHAPTER II

DEVELOPMENT OF THE OBJECT-PICTURE IN THE CASE WHERE OBJECTS ARE DISPOSED UPON A SINGLE OPTICAL PLANE . . . 10

SECTION I. Macroscopic Objects: *Introductory—Apparatus required for the subjoined experiments—Arrangements for obtaining the different types of illumination required for the development of the outline and object-picture—Experiments on the production of outlines—Deductions from the above experiments—Manner in which outlines are produced and effaced—Influence exerted by the refractive index of the enveloping medium upon the development and suppression of outlines.*

SECTION II. Microscopic Objects: *Introductory—Method of making the apparatus required for the subjoined experiments—Experiments with glass spherules and cylinders—Experiments with blood films—Comment on the above experiments—Experiments with stained blood films—Influence exerted by the refractive index of the enveloping medium upon the character of the object-picture.*

CHAPTER III

DEVELOPMENT OF THE OBJECT-PICTURE IN THE CASE WHERE THE ELEMENTS, WHICH ARE VIEWED BY THE UNAIDED EYE, OR, AS THE CASE MAY BE, BY THE MICROSCOPE, ARE PILED ONE UPON THE OTHER. 27

Introductory—Experiments on the conditions which determine the transmission of light through a number of superposed objects—Familiar examples of the dependence of opacity upon refraction and reflection of light from translucent elements, and of transparency upon the abolution of such refraction and reflection—On the dependence of opacity in the case of microscopical preparations upon the refracting and reflecting properties of the constituent elements—Deductions from the foregoing experiments—Method by which refraction and surface reflections may be abolished, and transparency secured, in the case where the interstices of a tissue are already filled in with water.

CHAPTER IV

PAGE

ON THE PRINCIPLES WHICH GOVERN THE STAINING OPERATIONS WHICH ARE UNDERTAKEN FOR THE PURPOSE OF DEVELOPING A MICROSCOPIC COLOUR-PICTURE 33

Introductory—Fundamental principles of staining—Chemical properties, classification and solubilities of the anilin dyes—Elementary conceptions with regard to the chemical properties of the histological elements of the animal body and their interaction with anilin dyes—Illustrative experiments—Importance of methods of differential staining considered as methods by which microscopical elements can be brought into view and identified.

CHAPTER V

ON THE RESTRICTIONS WHICH HAMPER THE MICROSCOPIST IN THE MATTER OF THE SELECTION AND DEVELOPMENT OF THE STAGE-PICTURE; ON THE DEFECTS AND SOURCES OF FALLACY WHICH ATTACH TO THE MICROSCOPIC OUTLINE-PICTURE AND MICROSCOPIC COLOUR-PICTURE RESPECTIVELY; AND ON THE LIMITATIONS WHICH ARE IMPOSED UPON MICROSCOPIC ACHIEVEMENT BY THE INADEQUATE REPRESENTATION OF THE OBJECT IN THE STAGE-PICTURE 39

Introductory—On the restrictions which hamper the microscopist in the matter of the selection and development of his stage-picture—On the disadvantages and imperfections and fallacies which attach to the microscopic outline-picture—On the disadvantages and imperfections and fallacies which attach to the microscopic colour-picture—General question of the limitations which are imposed upon microscopic achievement; and appraisement of the relative importance of each of the limitations which come into consideration.

PART II

THE MICROSCOPIC IMAGE AND ITS DEVELOPMENT

CHAPTER VI

PAGE

IMAGE FORMATION BY THE SIMPLE APERTURE 51

Function of the aperture in the formation of images—Conditions which determine the scale and brilliancy of the simple aperture image—Conditions which govern definition and resolution in plan in the case of the simple aperture image—Resolution in depth—Obstructive interference—Summary of the salient features of simple aperture images—Dilemma of the simple aperture image.

CHAPTER VII

ON IMAGE FORMATION BY THE LENS-ARMED APERTURE . . . 62

Preliminary—Resemblances between the image furnished by the "simple" or "vacant" aperture and the image furnished by the "lens-armed" aperture—Differences between the simple aperture image and the lens image—Separate definition of elements placed side by side upon the same object plane—Separate definition of elements placed one behind the other on different focal planes—Imaging of elements in the object which are concealed from direct view by intervening obstacles.

Conception of a "VISTA."

"POINT VISTAS"—Dimensions which come into consideration in the case of a "point vista" and method of measuring and expressing these dimensions—"Angular aperture"—"Numerical aperture"—Examples of the method of calculating angular aperture and numerical aperture—Connexion between the configuration of a vista and the quality of the image obtained—Relation between aperture and the greater or less brilliancy of the point image—Relation between the aperture and the greater or less sensitiveness of the image to alterations of the focussing adjustment—Relation between the aperture and the greater or less liability of the image to occultation by intrusive elements—Conception of interpenetrating point vistas—Influence of the aperture upon the definition of the focal images in the case of intrusive vistas.

"SURFACE VISTAS"—Configuration of (a) the simple magnifying vista, of (b) the simple minifying vista, and (c) of the vista in which magnification is followed by minification—Dimensions which come into consideration in the case of surface vistas—Relation between the configuration of the surface vista and the scale of the image—Examples of the application of the formulæ for the determination of the magnification of the image—Extent of the object field which is imaged—Examples of the application of the formula for determining the extent of the object field—Quality of the image—Influence exerted by the aperture of the vista upon resolution in plan—Influence exerted by the aperture of the vista upon resolution in depth—On the aperture of the vista as a factor which determines the extent of the plane of origin occulted by an intrusive element and the more or less satisfactory representation in the image of partially eclipsed elements.

Conception of a VISTA as a sequence of radiant planes.

Conception of a CATENA OF VISTAS—Image formation in a catena of surface vistas—Law governing the inversion of the image in a catena of surface vistas—Methods of measuring the scale of the image obtained on the terminal plane of a catena of vistas—Determination of the scale of the terminal image of a catena of vistas by dividing the numerical aperture of the opening angle (N.A.) by the numerical aperture of the closing angle (n.a.)—On the imaging of the successive optical planes of the original vista—Imaging of the apertural plane of an antecedent vista in the apertural planes of all the following vistas—Preliminary indications of the practical applications of the fact that the field upon which is imaged the apertural plane of the antecedent vista corresponds in area and position with the apertural plane of the

PAGE

following vista—Exploitation of the circumstance that the sectional measurement of the image of the aperture of the antecedent furnishes the sectional measurement of the Ramsden disk of the following vista—Exploitation of the circumstance that in a catena the apertural plane of every antecedent vista is imaged and available for scrutiny in the Ramsden disk of the terminal vista—Association of progressive restriction of the aperture with cumulative magnification, and preliminary indications of the significance of this progressive restriction of the aperture in connexion with the development of a critical image, and the limit of microscopic resolution.

CHAPTER VIII

ON THE DEFECTS WHICH ARE INTRODUCED INTO THE IMAGE BY SPHERICAL AND CHROMATIC ABERRATION IN THE LENS 95

Introductory—Distortion by spherical aberration—Method of correcting the distortion produced by spherical aberration—Distortion due to chromatic aberration—Method of correcting for chromatic aberration.

CHAPTER IX

ON THE DEFECTS INTRODUCED INTO THE IMAGE BY DIFFRACTION OCCURRING IN THE APERTURE OF THE LENS 101

Introductory—Brief exposition of the undulation theory of light—Conception of the vista modified in such a manner as to bring it into accord with the more adequate conception of the light distribution in the lens image now achieved, and signification to be attached to the term "antipoint"—Antipoint of a luminous point viewed through a restricted circular opening—Antipoint of a luminous point viewed through an aperture which is restricted along one diameter—Antipoint pattern of a file of points—Antipoint pattern of a luminous surface—Dependence of the antipoint pattern upon the brilliancy of the source of light, and distinction between "theoretical" and "conspicuous" antipoint. Law which governs the dimensions of the "theoretical," as distinguished from the "conspicuous" antipoint—Practical importance of the antipoint in connexion with the microscopic image—On the central elements of the antipoint and on the loss of resolution entailed by the overlapping of the false disks of adjoining antipoints—On the outlying elements of the antipoint considered in relation to the development of "spurious detail" in the image—Development of "intercostal spots"—Antipoint patterns furnished by diffraction gratings—Preliminary experiments with the diffraction grating—Dimensions of the antipoint pattern furnished by a grating—Examples, and verification of the formula for the dimensions of the antipoint furnished by a grating—Development of "intercostal lines" in such a form as to produce doubling or trebling in a periodical ruling.

APPENDIX I.—*On Abbe's theory of microscopic vision and on the effect of diffraction occurring in the object plane and opening limb of the vista.*

APPENDIX II.—*On the development of the median intercostal line considered as a fiduciary phenomenon upon which accurate adjustment may be made in making optical measurements.*

CHAPTER X

IMAGE FORMATION IN THE CASE WHERE THE OBJECT IS VIEWED BY THE UNAIDED EYE 132

Introductory—Inversion—Scale of the retinal image—Psychological factors involved in the estimation of the scale and disposition of the retinal image—Quality of the image furnished by the eye—Resolving power of the normal eye—Factors upon which the quality of the image depends—Numerical aperture of the eye—On the effect exerted upon the retinal image by a reduction of the numerical aperture of the eye—Significance of the results arrived at in the foregoing experiments in relation to the question of the limitation of resolution by the aperture of the objective—Question of the existence of an absolute physical limit to the resolving power of the eye, irrespectively of such limitations as may be imposed by the structure of the retina—Projection picture of the pupillary aperture and entoptic picture.

CHAPTER XI

IMAGE FORMATION IN THE CASE WHERE AN OBJECT IS VIEWED THROUGH A SIMPLE MICROSCOPE 143

Introductory—Inversion—Disposition of the reinforcing lens in the vista—Magnification—Methods of measuring the scale of the image obtained in the case where a convex lens is used as a doublet with the optical system of the eye—Description of the eikonometer and procedure for measuring the magnifying power of a simple microscope by its aid—Quality of the image—Effect of artificially shutting down the numerical aperture of the simple microscope—Delimitation of the field of the image.

CHAPTER XII

PAGE

IMAGE FORMATION IN THE CASE WHERE THE OBJECT IS VIEWED THROUGH THE COMPOUND MICROSCOPE. 150

Introductory—Optical system of the compound microscope—Detailed consideration of the microscope stage and eye-piece diaphragm (objective and field-lens) vista—Magnification achieved in this vista and procedure for measuring this magnification—Measurement of the extent of object field which is imaged—Quality of the image furnished by the objective—Eye lens and eye vista—Magnification achieved in this vista and methods for the measurement of this magnification—Quality of the image furnished by this vista—Vista of the substage condenser—Scale and quality of the image furnished by the condenser—Catena of the apertural planes of the microscope—Configuration and course of the beams in the catena in question—Scale of the image furnished in the Ramsden disk of the eye lens—On the primary catena of the microscope considered as a whole and on the terminal image which is furnished by this catena—Procedure for measuring the magnifying power of the microscope by means of the eikonometer—Procedure for measuring the magnifying power of the microscope by the aid of a stage micrometer and diffraction grating—Procedure for arriving at the magnification by dividing the numerical aperture of the opening limb of the objective and field-lens vista by the numerical aperture of the closing limb of the eye lens and eye vista—Quality of the terminal image, and on the superimposition upon this of an entoptic picture.

CHAPTER XIII

ON THE OPTICAL ELEMENTS OF THE MICROSCOPE CONSIDERED SEPARATELY 168

Mirror—The substage diaphragm—The substage condenser—Focussing of the substage condenser—Supplementary points in connexion with the focussing of the condenser—Centring adjustment of the substage condenser—Purpose of the centring adjustment of the condenser—Method of employing the condenser as an immersion system—Optical properties of the condenser—Focal length—Numerical aperture of the condenser—Measurement of the numerical aperture of the condenser—Method of obtaining from the Abbe condenser a wider beam than that which corresponds to its numerical aperture—Corrections of the condenser, and question of the relative advantages of the "Abbe" and "achromatic" condenser—Method of testing the corrections of the condenser—Slide and cover-glass and media interposed between condenser and objective—Influence exerted upon the numerical aperture of the transmitted beam by the optical media interposed between condenser and objective—Distortion of the configuration of the closing limb of the condenser vista and the opening limb of the objective vista by the slide and cover-glass and mounting medium—Distortion of the closing limb of the condenser vista by the interposition of the slide in the case where the microscopic object is mounted in air and the condenser is unimmersed—Distortion of the opening limb of the objective vista by the interposition of the cover-glass in the case where the microscopic object is mounted in air and the objective is unimmersed—The objective—Classification into dry and immersion objectives, and advantages of the latter in the case where highly magnifying systems are employed—Method of employing an oil-immersion objective—Focussing of the objective—Centring of the objective—Optical properties of the objective—Focal length and magnifying power of the objective—Method of measuring the focal length of the objective—On the numerical aperture of the objective and its influence upon the quality of the microscopic image—Measurement of the numerical aperture of the objective—Principle of the Abbe apertometer—Procedure for measuring the N.A. of the objective by means of the Abbe apertometer—Procedure for measuring the numerical aperture of the objective without recourse to the apertometer—On the accurate figuring and correction of the objective, and on the influence exerted by these factors upon the quality of the microscopic image—Corrections of the objective—Correction collar—Preliminary considerations in connexion with the testing of the accuracy of the figuring and the adequacy of the corrections—Procedure to be adopted in connexion with the proving of an objective—Consideration of the suitability of diatoms as test-objects for the proving of objectives—Employment of stained film preparations of bacteria (a) *as test-objects for the general accuracy of the figuring and corrections,* (b) *as special test-objects for proving the chromatic corrections, and* (c) *as test-objects for proving the flatness of the image field—Objective changers—Barrel of the microscope and draw tube—Width of the barrel—The ocular—Determination of the magnifying power of the ocular—Corrections of the ocular.*

CHAPTER XIV

PAGE

ON THE INSTRUMENTAL ADJUSTMENTS REQUIRED FOR THE ACHIEVEMENT OF DARK GROUND ILLUMINATION AND STEREOSCOPIC ILLUMINATION IN PARTICULAR IN CONNEXION WITH WIDE-ANGLED HIGH-POWER OBJECTIVES 208

Introductory, and experiments illustrating the influence of the N.A. of the objective on the type of picture imaged in the microscope—Problem of achieving dark ground illumination in connexion with both wide and narrow-angled objectives—Two distinct schemes of illumination can be exploited for the purpose of achieving dark ground illumination—Method of achieving dark ground illumination in connexion with narrow-angled low-power objectives by the "arrangement by which the preparation is illuminated by solid, and imaged by hollow beams"—Method of achieving dark ground illumination in connexion with wide-angled high-power objectives by the "arrangement by which the preparation is illuminated by hollow, and imaged by solid beams"—Method of Siedentopf and Zsigmondi—Method of achieving dark ground illumination in connexion with wide-angled high-power objectives by the "arrangement by which the preparation is illuminated by solid, and imaged by hollow beams."

CHAPTER XV

ON THE ADJUSTMENTS REQUIRED FOR THE ACHIEVEMENT OF A CRITICAL IMAGE 218

Introductory—Necessity for the regulation of the source of illumination—Suggestions for the avoidance of the difficulties which are associated with the employment of an extended source of illumination—Disadvantages which attach to Nelson's method of evading these difficulties—Methods of reducing the dimensions of the light source—Importance of centring the condenser—Principles in accordance with which the working aperture of the condenser ought to be regulated—Method of regulating the substage arrangements in such a manner as to give us a system of homogenous beams of the greatest possible numerical aperture—Choice of objective—Adjustments which may be required for the purpose of obtaining the full benefit of the optical corrections of the objective—Elimination of bubbles in the immersion medium and of any obtruding obstacles which may be disposed on the upper or lower face of the condenser or objective—Choice of ocular—Eperiments exhibiting the effect which is exerted on the microscopic image by the contraction and opening up of the beams which are delivered by the microscope to the eye.

CHAPTER XVI

ON THE QUESTION AS TO WHETHER THERE ARE DEFINITE LIMITS IMPOSED UPON MICROSCOPIC VISION AND RESOLUTION; AND ON THE REAL NATURE OF THE LIMITATIONS ENCOUNTERED IN CONNEXION WITH THE EMPLOYMENT OF HIGH MAGNIFICATIONS . 229

Introductory—Theoretical limit of resolution—Question as to whether the limit of Helmholtz is the untransgressible limit of resolution—Question as to the real nature of the limit of resolution which is encountered by the microscopist in connexion with the employment of high magnifications—Is it possible to advance to a higher limit of resolution than that attained by the present optical arrangements?—On Gordon's method of opening up the beam before it is delivered by the microscope to the eye—Appraisement of this achievement—Question as to whether it opens up a prospect of an advance towards increased resolving power.

DESCRIPTION OF PLATES

PLATE I. (*Frontispiece*).

A. Radiant field consisting of white disk disposed on a black ground, with inset figure showing that a dark margin is developed round a glass spherule when placed in position against such a field. (*Dark outline picture.*)

B. Radiant field consisting of black disk disposed on a white ground, with inset figure showing that a bright margin is developed round a glass spherule when placed in position against such a field. (*Dark ground illumination.*)

C. Radiant field consisting of a homogeneous white surface, with inset figure showing that a glass spherule is only very lightly outlined when placed in position against such a field. (*Suppression of outline picture.*)

D. Radiant field consisting of a blue disk disposed upon a red field, with inset figure showing that a spherule placed in position against such a field is seen as a blue object delineated by a red outline (*illumination by differentially coloured light*).

E. Series of extemporized substage stops made in the manner described in Cap. II., Sect. II., subsect. 2., p. 20.

a. dark outline picture ; *b.* bright outline picture ; *c.* picture in relief ; *d.* pure colour picture ; *e.* colour and outline picture (*vide* pp. 3–4 and p. 12).

PLATE II. (*facing page* 20).

a. Glass spherule, mounted in Canada balsam and illuminated by the fully open focussed condenser in such a manner as to obliterate the delimiting outline (*vide* p. 21, Exp. 3 ; p. 99, Exp. 1 ; and p. 174).

b. Glass spherule, delineated by a bright outline upon a dark ground. (*Dark ground illumination.*) The object, which is mounted in water, is illuminated by hollow, wide-angled beams furnished by a wide-angled condenser fitted with a central spot stop, as shown in Figs. 75 and 90, and is viewed with a narrow-angled objective.

c. Glass spherule delineated by a dark outline upon a bright ground. The object is mounted in water, is illuminated by narrow-angled beams and is viewed with a narrow-angled objective. (*Dark outline picture.*)

d. Film preparation of tubercle bacilli, differentially stained red, mixed with other bacteria which are stained blue. Preparations such as this here in question serve as a test for chromatic aberration in the microscope (*vide* p. 99, Exp. 2).

e. Reproduction of a drawing of a portion of a test diatom (triceratium). This serves to illustrate the difference between the " funda-

mental" and the "derivative" pattern (*vide* p. 8, Exps. 1 and 2, and footnote).

f. Glass spherule delineated by a dark outline upon a blue field. The object is mounted in water and is illuminated by narrow-angled beams which have been transmitted through the central area of the parti-coloured stop shown on Plate I, Fig. E, 3. The outline appears yellowish by colour-contrast when viewed through the covering tissue-paper (*vide* pp. 8–9 and p. 21, Exps. 4 and 6).

g. Glass spherule delineated by a dark outline upon a red field. The object is mounted in water and is illuminated by narrow-angled beams which have been transmitted through the peripheral area of the parti-coloured stop, shown in Plate I, Fig. E, 3, placed under a narrow substage diaphragm. The outline appears greenish by colour-contrast when viewed through the covering tissue-paper (*vide* pp. 8–9).

h. Glass spherule delineated by a red margin on a blue field. The object is mounted in water and is illuminated by wide-angled beams which have been transmitted through the parti-coloured stop placed centrally under a widely-open focussed condenser.

i. Semi-diagrammatical representation of a piece of blotting paper of which one half (the left half) is mounted in oil, while the other (right) half is mounted in air, the whole being illuminated by narrow-angled beams. On the left side, owing to the abolition of refraction and reflection by the filling in of the interstices of the paper by oil, the preparation has become transparent and the fibre of the paper has been rendered invisible. On the right side, where the interstices of the paper are filled in with air, refraction and reflection are preserved, with the result that the preparation is opaque and that the constituent fibres are delineated by hard dark outlines (*vide* pp. 29–30, Exps. 1–7).

j. Representation of the same object similarly mounted and similarly illuminated except in the respect that the light has been transmitted through a red light filter (*vide* pp. 29–30, Exps. 1–7).

k. Glass wool delineated by dark outlines upon a blue field. The filaments are here mounted in water and are illuminated by narrow-angled beams transmitted through a blue light filter (as shown in Plate XI, Fig. 2) and are viewed with a narrow-angled objective. The dark outlines appear yellow by colour contrast when viewed through the covering tissue-paper (*vide* pp. 8–9 and p. 177, Exp. 2).

l. Glass wool seen against a purple ground. The filaments are here mounted in water and are illuminated by wide-angled beams furnished by a fully open condenser and a parti-coloured stop (as shown in Plate XI, Fig. 1), and are viewed with a wide-angled objective (*vide* p. 210, Exp. 2).

m. Glass wool delineated by red outlines on a blue field. The filaments are here mounted in water, are illuminated by wide-angled beams furnished by a fully-open condenser and a parti-coloured stop (as shown in Plate XI, Fig. 1), and are viewed with a narrow-angled objective (*vide* p. 210, Exp. 2, and p. 155, Exp. 2). The red outlines, when viewed through the covering tissue-paper, appear yellowish by colour contrast to the blue field.

PLATE III (*facing page* 22).

a. A film preparation of blood in which the elements are delineated by dark outlines upon a bright field. (*Dark outline picture.*) The

preparation is mounted in air, is illuminated by narrow-angled beams and is viewed with a narrow-angled objective (*vide* p. 22, Exp. 1; p. 41 (b); and p. 42, Exp. 2).

b. A film preparation of blood in which the elements are delineated by bright outlines upon a dark field. (*Dark ground illumination.*) The preparation is mounted in water, is illuminated by hollow wide-angled beams (furnished by a wide-angled condenser fitted with a central spot stop as shown in Figs. 75 and 90), and is viewed with a narrow-angled objective (*vide* p. 23, Exp. 2).

c. Film preparation of blood seen as a picture in relief. The preparation is mounted in air, is illuminated by such a system of obliquely incident beams as is shown in Fig. 76, and as is obtained by the arrangement shown in Plate XII, Fig. 1 (*vide* p. 23, Exp. 4).

d. Film preparation of blood in which the elements are visible only in so far as they are coloured (colour picture). The preparation is illuminated by a focussed fully-open wide-angled condenser in the field of which is the window bar shown in the upper part of the figure (*vide* p. 23, Exp. 3; p. 99, Exp. 1, and p. 174).

e. Film preparation of blood in which the elements are differentially stained by Leishman's stain (*vide* p. 24).

f. Film preparation of blood stained diffusely with eosin (see p. 36, Exp. 6).

g. The same preparation partially discolourized by the agency of a weak alkali (see p. 36, Exp. 5).

h. The same preparation after further staining with methylene blue (see p. 36, Exp. 7).

PLATE IV (*facing page* 28).

A. Diagrammatic representation of a glass cell filled with transparent highly refractive spherules lying in air. The opacity of the contents of the cell is seen to be due to the fact that the rays which here pass up through the floor of the cell are diverted from their course by refraction and reflection (*vide* pp. 27 and 28, Exps. 1–4).

B. Diagrammatic representation of the same cell and spherules lying in a highly refracting enveloping medium. The transparency of the contents of the cell is seen to be due to the fact that the rays here pass upwards through the cell without being turned aside from their course by reflection or refraction (*vide* pp. 27–28, Exps. 1–4).

PLATE V (*facing page* 42).

Fig. 1. Diagram showing manner in which illumination by differentially coloured light is achieved in the case of a spherule placed in position as in Plate I D, against a parti-coloured radiant field (*vide* p. 17 (*d*)).

Fig. 2. *Vide* p. 42, Exp. 2 and comment.

Fig. 3. *Vide* p. 42, Exp. 2 and comment.

PLATE VI (*facing page* 78).

A and *B*. Diagrams showing influence of aperture upon the definition of the focal images in the case of interpenetrating point vistas derived from radiant points placed one behind the other (*vide* p. 78, subsects. 17 and 18).

C and *D*. Diagrams showing the influence of aperture upon the definition of the images in the case of interpenetrating surface vistas derived from radiant planes placed one behind the other (*vide* p. 82, subsect. 28).

PLATE VII (*facing page* 98).

Fig. 1. Diagram showing the effect of chromatic aberration in the case where an isolated beam passes through an uncorrected lens (*vide* p. 98, subsect. 4).

Fig. 2. Diagram showing the effect of chromatic aberration in the case where light derived from differently coloured radiant points passes through an uncorrected lens (*vide* p. 99 [2]).

Fig. 3. Diagram showing the optical anatomy of the apertural plane of the objective. A.P. Apertural plane of objective combination where the pencils of parallel rays intersect to form luminous points which radiate a mixture of blue and red rays (*vide* p. 155, including Exps. 1 and 2).

Fig. 4. Diagram showing the optical anatomy of the apertural plane (Ramsden disk) of the eye lens. E.L., eye lens; A.P., Ramsden disk where the pencils of parallel rays intersect to form luminous points which radiate a mixture of blue and red rays. (*vide* p. 158, including Exps. 1 and 2).

PLATE VIII (*facing page* 106).

Fig. 1. Diagram of a dioptric beam supplemented by a system of diffracted beams. The central beam (unshaded) represents the dioptric beam; the lightly shaded beams, the *luminiferous* diffracted beams; the darkly shaded beams, the *caliginiferous* diffracted beams. A, point of origin of the beam; B, aperture of the lens seen in optical section; C., diffraction pattern (antipoint) which represents in the image the luminous point A; C′, optical section of the antipoint C. (*vide* pp. 106–107).

Fig. 2. Antipoint of a luminous point as seen through two slit openings. A., radiant object point; B., aperture; C., antipoint (*vide* p. 108, Exp. 2).

Fig. 3. Diagram showing the antipoint pattern of a circle seen through a restricted circular aperture. A, object line; B, circular aperture; C, antipoint pattern. When viewed through the covering tissue-paper, the antipoint pattern consists only of the principal circle and an intercostal point (*vide* p. 108).

PLATE IX. (*facing page* 108).

Fig. 1. Diagram showing the antipoint pattern of a straight line seen through a linear aperture. A, object line; B, linear aperture; C, antipoint pattern (*vide* p. 108).

Fig. 2. Diagram showing antipoint pattern corresponding to a straight line seen through a narrow circular aperture. A, object line; B, circular aperture; C, series of antipoints corresponding to the file of point which radiate light through the aperture (*vide* p. 108).

Fig. 3. Diagram showing antipoint of a disk seen through a linear aperture. A, object; B, aperture; C, antipoint pattern (*vide* p. 109).

PLATE X (*facing page* 158).

Figs. 1 and 2. Photographs showing the Ramsden disk of eye lens as seen on looking down upon the microscope from a distance of 10 or more inches. A, Ramsden disk. The smaller size of the Ramsden disk in Fig. 2 stood in this instance in relation with a narrowing of the substage diaphragm (*vide* pp. 90–92, 158, 160–161).

Fig. 3. Drawings of the Ramsden disk of the eye lens as seen by placing a pocket lens in front of the eye and bending over the microscope.

A. This drawing corresponds to Fig. 1, *supra*. Here in conformity with the fact that the aperture of the objective is fully filled in by the beams which proceed from the microscope stage, the Ramsden disk corresponds to the image of the whole back surface of the objective. The black dots seen in the image correspond to particles of dust which have lodged upon the upper surface of the back lens of the objective (*vide* p. 93).

B. The drawing corresponds to Fig. 2, *supra*. Here in conformity with the fact that the beams which proceed from the stage of the microscope fill in the centre portion only of the aperture of the objective, the Ramsden disk corresponds to an image of the centre of the back of the objective and is enveloped by a half-shaded area which corresponds to an image of the upper part of the substage diaphragm.

C. The drawing corresponds to Fig. 2, *supra*, with the difference that the centre of the substage diaphragm is here out of allineation with the centre of the objective (*vide* p. 180 and p. 222).

Figs. 4 and 5. Drawings of the projection picture of the pupillary aperture (entoptic picture) which comes into view when a homocentric beam radiates into the pupil.

In Fig. 4 are to be noted (*a*) the granular mottling of light and shade over the whole extent of the luminous disk (this is caused by want of homogeneity in the ocular media); (*b*) the two bright points, each with its system of diffraction rings (these correspond to tears upon the surface of the cornea); (*c*) the more centrally placed diffraction patterns corresponding to intralenticular opacities; (*d*) the spiked contour which corresponds to the pupillary margin of the iris; and (*e*) the double bright and dark contour caused by diffraction of the light at the edge of the pupillary diaphragm.

In Fig. 5 are superadded the shadow of two eyelashes (each with its diffraction bands) and a broader, mottled, horizontally-disposed shadow with diffraction bands corresponding to a ridge of debris swept up by the eyelid from the surface of the cornea.

PLATE XI (*facing page* 176).

Figs. 1 and 2. Diagrams showing that when a parti-coloured red and blue screen such as is shown on Plate I, E, 3, is employed in the substage beams of mixed blue and red rays are brought to focus by the condenser.

In Fig. 1, where the condenser has been focussed upon the upper surface of the slide, both red and blue rays would radiate into the objective from the microscopic preparation.

In Fig. 2, where the principal focal plane of the condenser lies below the level of the microscopic preparation, this last is illuminated by the blue rays only (*vide* pp. 176–177).

PLATE XII (*facing page* 180).

Fig. 1. Photograph of arrangements of the substage which will furnish a system of luminous points radiating light in each case in the form of a narrow solid cone along an axis disposed obliquely to the optical axis of the microscope in such a manner as to give a stereoscopic picture (*vide* pp. 172–173, and Plate III, *c*).

Fig. 2. Zeiss objective changer (*vide* p. 205).

Fig. 3. Abbe's apertometer. A, plan ; B, elevation (*vide* p. 198).

Fig. 4. Dry objective fitted with correction collar (*vide* p. 201).

PLATE XIII (*facing page* 186).

Diagram showing the automatic colour correction which occurs where rays derived from adjoining uncorrected beams intersect to form nodal points (*vide* p. 186).

PLATE XIV (*facing page* 218).

Diagram showing the conditions which obtain where an extended radiant field is employed as the source of light. (With a view to facilitating the elucidation of these conditions, a parti-coloured red and blue screen serves in this and the succeeding diagrams in each case as the source of light.)

A. Diagrammatic representation of the image which is formed in the diaphragm of the ocular. The red disks here represent the antipoints corresponding to the radiant points in the central portion of the object field which send their light in the form of wide unmutilated beams symmetrically through the aperture of the objective ; the blue elliptical figures on the periphery which extend inwards over the image field represent the elliptical antipoints corresponding to the radiant points in the periphery of the object field which send through the aperture of the objective beams which are cut down in an unsymmetrical manner by the edges of the objective mount.

B. Diagrammatic representation of the apertural plane of the objective where the blue and red beams intersect to form nodal points which radiate as shown in Plate VII., Fig. 3 (A.P.), a mixture of blue and red rays. To be noted here is also the fact that the apertural plane of the objective is fully filled in by the transmitted beams (*vide* p. 219).

PLATE XV (*facing page* 220).

Diagram showing the conditions which obtain when, in conformity with Mr. Nelson's suggestion, the substage condenser is partially closed, with a view to avoiding that fogging of the image which is ordinarily associated with the employment of an extended radiant field. The general scheme of the diagram is the same as that employed in connexion with Plate XIV. To be noted is the circumstance that, in conformity with the narrowing of the substage diaphragm and the correspondingly diminished width of the beams which radiate into the objective, the apertural plane of the objective B is only partially illuminated. As a further result, the radiant points upon the stage are represented in the

image by antipoints which are larger than those which correspond to the full aperture of the objective (*vide* pp. 220–221).

Owing to an oversight the blue vistas which ought properly to have been represented as incurved vistas passing into the objective have here been allowed to stray afield. In association with this, the antipoints on the periphery of the image-field (A) ought to have been coloured blue instead of red.

PLATE XVI (*facing page* 222).

Diagram showing the conditions which obtain when, in accordance with Gordon's suggestion, the dimensions of the radiant field are severely restricted. The general scheme of the diagram is the same as that employed in connexion with Plate XIV. To be noted is the circumstance that, in conformity with the fact that the full aperture of the condenser is employed, the radiant points on the stage now furnish beams which fill in the whole apertural plane of the objective (B) furnishing on the now only partially illuminated image plane of the objective (A) the smaller antipoints which correspond to that full aperture (*vide* pp. 220–221).

PLATE XVII (*facing page* 224).

Diagram showing the conditions which obtain where the objective is out of allineation with the condenser. The general scheme of the diagram is the same as that employed in Plate XIV. To be noted is the circumstance that, owing to the unsymmetrical manner in which the beams are cut down in the course of their passage through the objective, they are represented in the image plane by ellipitical antipoints (*vide* p. 222).

PLATE XVIII (*facing page* 236).

Photographs exhibiting the impediments to microscopic resolution which are referable to diffraction and obfuscation and the improvement which is effected in the highly magnified microscopic image by the employment of Gordon's device for opening up the beam in the image plane of the objective.

Figs. A, B, C are photographs obtained by the aid of Gordon's microphotographic apparatus used in conjunction with his tandem microscope giving upon the photographic plate an actual magnification of circ. 2,500 diameters and a magnification upon the retina that would be conventionally described (*vide* Cap. X, subsect. 4) as a magnification of about 7,500.

Fig. A. Group of typhoid bacilli imaged by Gordon's tandem microscope employed without the ground glass screen. To be noted are the shadows and diffraction patterns of small particles of dust lying on the eye lens (*vide* pp. 93–94 ; pp. 227–228 ; p. 233 and p. 236). We have here the factor which is contributed by the microscope to the obfuscation and diffraction which mar the highly magnified microscopic image.

Fig. B. Photograph of the central portion of the entoptic picture depicted in Plate X, Fig. 5 (*vide* pp. 140–142, p. 167, p. 228, p. 233, and p. 237). This represents the factor which is contributed by the eye to the obfuscation and diffraction which mar the highly magnified microscopic image.

Fig. C. A composite photograph of Fig. A and Fig. B.

Owing to an accidental inversion of one of the plates, the right side of Fig. B is in the composite superimposed upon the left side of Fig. A.

This represents the combined contribution of the microscope and the observer's eye to the obfuscation and diffraction which mar the highly magnified microscopic image.

Fig. D. Group of typhoid bacilli imaged by Gordon's tandem microscope employed with the ground glass screen at rest. We have here the highly magnified microscopic image relieved from the blemishes (seen in C) which are referable to obfuscation and diffraction, but marred by the superimposed magnified image of the grain of the screen (*vide* pp. 236–237).

Fig. E. Group of typhoid bacilli imaged by Gordon's tandem microscope employed with the ground glass screen in motion. The highly magnified microscopic image has here been relieved both from the blemishes contributed by obfuscation and diffraction and from the superadded blemishes referable to the superimposition upon the picture of the magnified image of the grain of the screen.

Part I.

THE OBJECT PICTURE AND ITS DEVELOPMENT.

CHAPTER I.

GENERAL CONSIDERATIONS WITH REGARD TO THE OBJECT PICTURE.

Definition of the object picture—Classification of pictures as outline pictures, colour pictures, outline and colour pictures, and pictures in relief respectively—Preliminary consideration of the respective advantages and disadvantages of the different varieties of pictures enumerated above—Advantages and disadvantages of the different varieties of pictures from the point of view of the disclosure of objects which (by reason of their minuteness or distance) subtend only a small angle at the retina—Advantages and disadvantages of the different types of pictures from the point of view of the disclosure of the configuration of the object in plan and relief—Advantages and disadvantages of the different types of pictures from the point of view of the disclosure of the internal structure of the object, Advantages and disadvantages of the different types of pictures from the point of view of their greater or less liability to be vitiated by fallacy.

1. Definition of the object picture.

We may designate by the term *object picture* that system of radiant points which forms the original of the image which is produced on the retina. Where an object is viewed by the microscope, the term *stage picture* may conveniently replace the more generic term object picture.

2. Classification of pictures as outline pictures, colour pictures, outline and colour pictures, and pictures in relief respectively.

Object pictures may be classified in conformity with the following considerations :—

(*a*) Objects may be defined by outlines alone. They may, according to circumstances, be delineated by black outlines on a bright field, or by bright outlines on a dark field. The corresponding pictures may be spoken of as "*outline pictures.*"[1]

(*b*) Objects may be defined against a background by a difference of colour. The corresponding pictures may be spoken of as "*colour pictures.*"

[1] This is the type of picture which Koch has, not altogether felicitously, designated the *Strukturbild*—structure picture.

(*c*) Objects may be defined both by outlines and by a difference of colour. The corresponding pictures may be spoken of as *combined outline and colour pictures.*

The outline pictures and colour pictures spoken of in (*a*) and (*b*) may be distinguished from those here in question as *pure outline* or *pure colour pictures* respectively.

(*d*) Objects may come into view as patches of dark and bright, the former corresponding to prominences and illuminated surfaces (understanding here by illuminated surfaces, surfaces which radiate light to the eye), the latter to hollows and shaded surfaces (i.e. surfaces which radiate no light to the eye). The corresponding pictures may, according as we are dealing with uncoloured or coloured objects, be spoken of as *pictures in relief* or *pictures in colour and relief.*

Figs. *a*, *b*, *c*, *d* and *e*, Plate I, furnish examples of the *dark outline picture*, the *bright outline picture*, the *picture in relief*, the *pure colour picture*, and the *outline and colour picture* respectively. Further examples of the first three are furnished respectively by *a*, *b*, and *c*, Plate III ; further examples of *colour pictures* by *d*, *e*, *f*, *g*, and *h* of Plate III.

We have familar every day examples of *outline pictures* in outline drawings in black on white and white on black. Ordinary artists' paintings and coloured transparencies would come under our heading of *colour pictures.* Leaded stained glass windows, and the daubs produced in the nursery by the superposition of colour on outline drawings, would constitute *colour and outline pictures.* Photographs of statuary and of the surface of the moon, lit from the side in such a manner as to give a stereoscopic effect, furnish familiar examples of *pictures in relief.*

3. Preliminary consideration of the respective advantages and disadvantages of the different varieties of pictures enumerated above.

A comparison of the respective advantages and disadvantages of the different types of pictures enumerated above involves in each case an inquiry into the capacity of each picture :—

(*a*) For rendering the object visible when it subtends only a small angle to the eye ;

(*b*) For disclosing its configuration in plan and relief ;

(*c*) For making manifest, in the case when it is transparent, its internal structure.

(*d*) For furnishing an image which shall not be liable to erroneous interpretation.

We may consider the types of pictures above enumerated from these different points of view.

4. Advantages and disadvantages of the different varieties of pictures from the point of view of the disclosure of objects which (by reason of their minuteness or distance) subtend only a small angle at the retina.

The respective advantages and disadvantages of the different types of pictures from the point of view of the disclosure of elements which subtend a comparatively small angle at the retina will come clearly before the eye on turning to Plate I and setting up the book at progressively increasing distances from the eye. It will be seen that resolution will be lost in Figs. *a* and *b*, where the elements are delineated by outlines, sooner than in Figs. *c*, *d* and *e*, where the elements are represented respectively, by patches of light and shade, by patches of colour, and by patches of colour enclosed in outlines.

Consideration will bring out the reason of these differences.

In *outline pictures*, such as are furnished by Figs. *a* and *b*, the elements remain visible only so long as the diameter of the delineating outline subtends the necessary angle upon the retina.

In *pure colour pictures*, such as are shown in Fig. *d*, the elements remain visible as long as their total diameter subtends that necessary angle.

In the case of *outline and colour pictures* and in the case of *pictures in relief*, resolution will be lost somewhat sooner than in the pure colour picture by reason of the fact that the area of colour, or, as the case may be, of illuminated or shaded surface, is in each case less than the total area of the object.

What holds true in the case of these figures holds true also generally. It holds true in the case of the bull's eye of a distant rifle butt. Here an advantage in visibility is secured by the substitution of a solid bull's eye, which subtends a comparatively large angle upon the retina for a bull's eye of similar dimensions defined only by an outline. It holds true also in connexion with the microscopic work where objects such as bacteria, which subtend even under high magnification only a small angle at the eye, are to be brought clearly into view.

5. Advantages and disadvantages of the different types of pictures from the point of view of the disclosure of the configuration of the object in plan and relief.

Subject to the reservation which is made below with respect to the *combined outline and colour picture*, all the types of picture

which have been enumerated above may be said to define, in a satisfactory manner, the *configuration in plan* of the object.

In connexion with the combined colour and outline picture a difficulty may arise as to whether the proper contour is that which runs along the confines of the coloured area, or that which follows the perimeter of the outline. The nature of the difficulty will be clear to us if we call up before us the situation which would arise if it were proposed that craniometrical measurements should be made upon the heads of the saints as depicted in heavily leaded stained glass windows. We might here be in doubt as to whether the leaded outlines were to be excluded or included in the measurements.

In the matter of their capacity for disclosing *configuration in relief*, important differences emerge as between the different types of picture. Bright outlines upon a dark background furnish only an external system of exterior outlines, and give in conformity with this a picture entirely upon one plane. We have striking illustration of this in Figs. *b*, Plates II and III. The same defect attaches, though in a less conspicuous manner, to the *dark outline picture*. On comparing Fig. *a*, Plate III, with Fig. *c* on the same Plate, it will be recognized that the configuration in relief is only very incompletely brought out by a system of outlines. A *colour picture* discloses in a more adequate manner the configuration in relief. To take a concrete example the difference in the depth of the colour as between the centre and the periphery of the red corpuscles, as depicted in a *colour picture*, would provide a satisfactory clue to the interpretation of their shape. But best of all from the point of view of the disclosure of the configuration in relief is the picture in light and shadow, or, as we have called it, the *picture in relief*. Referring again to Fig. *c*, Plate III, we see that the concave shape of the red blood corpuscles is here directly enforced upon the attention.

6. Advantages and disadvantages of the different types of pictures from the point of view of the disclosure of the internal structure of the object.

When we come to realize, as we may here in anticipation, that *dark outline pictures* and *colour pictures* respectively as obtained with the microscope are in each case furnished by light transmitted through the object, while the *picture in relief* is obtained in part by the reflection of light from the external surfaces of objects, it is borne in upon us that the two former types of pictures will be appropriate for the disclosure of the internal structure as well as the configuration in plan, and that the latter type of picture will

disclose only the configuration in plan and relief—not the internal structure.

A comparison of the white blood corpuscles in Figs. *a*, *e* or *h*, Plate III, with the same corpuscles in Fig. *c* on the same Plate, will bring this point out clearly.

7. Advantages and disadvantages of the different types of pictures from the point of view of their greater or less liability to be vitiated by fallacy.

Reference may be made here to two of the more important fallacies which come into consideration.

A. Misprision of Focus. We may denote by the term *misprision of focus* the error we fall into when, misled by the sharpness of the image obtained, we erroneously assume that we are focussed upon the object, and that the image which is under consideration furnishes a correct representation of the disposition of the radiant points in the object. This fallacy is incident in particular in the microscopic outline picture, which is, as we shall see, a picture produced by reflection and refraction.

We lapse into the fallacy of misprision of focus.

(1) When we refer to one and the same optical plane elements which are in reality disposed upon different optical planes.

(2) When we mistake for the system of radiant points which is positioned in the object itself—we may speak of this as the *fundamental picture*—a system of radiant points—we may speak of this as the *phantom or derivative picture*—formed closer to the eye by the intersection of the rays proceeding from the radiant points aforementioned.

(3) When, by reason of a defect of accommodation, or in the case where a microscope is employed by reason of its being focussed on a plane lying on the further side from the object, a geometrical pattern is represented upon the retina by a pattern of diffusion discs which reproduces the original pattern somewhat as a negative does a positive.

The *first* variety of the fallacy of misprision of focus finds illustration—

(*a*) In the fact that two systems of lines disposed in reality upon separate horizontal planes may, in the case where they cross each other at right angles, be interpreted by the eye as constituting a system of squares ; and

(*b*) In the fact that oxalate of lime crystals present, as seen under a low magnifying power, the envelope-shaped appearance

which is represented in Fig. 1. Here the outlines which produce the characteristic stellate cross are in reality the delimiting outlines of two four-sided pyramids which project respectively upwards and downwards from the optical plane upon which the external outlines of the figure are disposed.

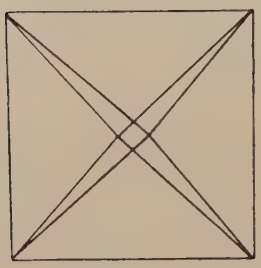


FIG. 1.
AN OXALATE OF CALCIUM CRYSTAL.

The *second* variety of the fallacy will find illustration in *Experiment* 2 below, and also in *Experiments* 4 and 5, Chapter V sub-sec. 3.

The *third* variety of the fallacy finds illustration in *Experiment* 1 described below.

Experiment 1. Turning to Plate II, Fig. *e*, dispose this first at a convenient distance from the eye, and then bring it nearer until its structure can no longer be seen in sharp focus. It will be observed that at the point at which accommodation gives out a process of inversion takes place, which gives us, instead of the pattern of dark discs included in white hexagons, a pattern of white discs included in dark hexagons.

Experiment 2. Take now a pocket lens before the eye and bend over the picture until the pattern comes into view in accurate focus. This done, carry the head closer to the paper until the picture is thrown out of focus. The pattern will now, as in the last experiment, be inverted. Now draw back from the paper beyond the point at which a focussed picture is obtained. The pattern of dark discs included in white hexagon will once more give place to a pattern of white discs included in dark hexagons.[1]

B. Subjective Colour Contrast. Whenever a coloured field is chequered in such a manner as to furnish areas on which the ground colour is subdued by shade, colour contrast phenomena will manifest themselves given that the patches in question radiate uncoloured light to the eye.

The practical importance of this fact in connexion with microscopic work will be recognized in a later stage. For the present it will suffice if the reader will turn to Plate II and will acquaint, or

[1] This figure may be employed as a convenient test for presbyopia or hypermetropia. Considered as a test for these conditions, it presents the great advantage that the critical sense of the patient will, provided that a perfectly regular geometrical figure is employed, be every whit as well satisfied by the unfocussed and "inverted" as by the duly focussed and "uninverted" image.

reacquaint, himself with the physiological phenomena which come here into consideration. He will find in Figs. *f* and *g*, Plate II, dark outlines laid down in the one case upon a blue in the other case upon a red field. Viewing these outlines through the covering tissue paper,[1] it will be observed that they assume under the influence of the uncoloured light which radiates from this into the eye respectively a yellow and a green tint. In a similar manner, the dark margins of the glass filaments represented in Fig. *k* assume, when looked at through the covering tissue paper, a distinctly yellow tint. These are only illustrations of the general law that where we have disposed upon a coloured field a darker area, this area will, under the influence of uncoloured light reflected from its surface, assume in each case a tint complementary to that of the enveloping field.

[1] The office performed by the tissue paper is to render uniform the illumination all over the field. It will be realized that the tissue paper is made up of bright outstanding elements—the strands of the paper, and of sunken shaded elements—the fenestrae between the fibres. In accordance with this it spreads over the picture a network of lights and shadows which has the effect of lighting up the darker portions of the picture and of toning down the brighter portions.

Any other device, which will in similar manner equalize the brightness, will be equally effective in bringing out the colour contrast effects. Thus, when, on a blackboard, we cover an area with coloured chalk and leave in the centre of this area a vacant patch, the imposition of a film of chalk powder on this empty space will bring out the colour contrast upon it.

In external nature mist, and in certain circumstances snow, take upon themselves the office of lighting up the shadows of the landscape and of throwing up the colour contrast phenomena into prominence. (*Vide* Author's paper on "Colour Shadows," *Nineteenth Century*, 1897.)

CHAPTER II.

DEVELOPMENT OF THE OBJECT PICTURE IN THE CASE WHERE OBJECTS ARE DISPOSED UPON A SINGLE OPTICAL PLANE.

SECTION I. **Macroscopic Objects:** *Introductory—Apparatus required for the subjoined experiments—Arrangements for obtaining the different types of illumination required for the development of the outline and object picture—Experiments on the production and effacement of outlines—Deductions from the above experiments—Manner in which outlines are produced and effaced—Influence exerted by the refractive index of the enveloping medium upon the development and suppression of outlines.*

SECTION II. **Microscopic Objects:** *Introductory—Method of making the apparatus required for the subjoined experiments—Experiments with glass spherules and cylinders—Experiments with blood films—Comment on the above experiments—Experiments with stained blood films—Influence exerted by the refractive index of the enveloping medium upon the character of the object picture.*

SECTION I.

EXPERIMENTS ON THE DEVELOPMENT OF THE OBJECT PICTURE IN THE CASE WHERE THE OBJECT IS VIEWED BY THE UNAIDED EYE, AND EXPLANATION OF THE MANNER IN WHICH OUTLINES ARE RESPECTIVELY PRODUCED AND EFFACED.

1. Introductory.

The conditions of visibility being, as we shall see as we go along, precisely the same in the case of the smallest objects made visible by the microscope as in the case of objects which can be seen by the unaided eye, we may conveniently begin by studying the conditions under which the object picture is produced in the case of such macroscopic objects.

2. Apparatus required for the subjoined experiments.

Cylinders and spheres of coloured and uncoloured glass will furnish convenient objects for experimentation. They will serve in some sort as prototypes of the coloured or uncoloured microscopic elements which are studied by the histologist and bacteriologist

(*a*) *Method of making the uncoloured cylinders and spherules required for the study of the object picture.*

An ordinary glass rod supplies the material for making the uncoloured objects we require.

Take a piece of glass-rod, hold it in a flame, preferably in a blow-pipe flame, until it fuses, then withdraw it from the source of heat and rapidly pull it out into a fine filament. The filament thus obtained will be a true cylinder. From such a filament an accurately figured sphere can be obtained by fusing its extremity in the fringe of a flame, allowing the melted glass to mould itself under the influence of surface tension.[1]

(*b*) Method of making the coloured glass cylinders and spherules. Select a pointed fragment of very deeply coloured window glass (avoiding ordinary ruby glass, which is only flashed with colour), and fuse the extremity of the fragment in the flame.

Having obtained in this manner a fused bead of coloured glass, seize this with a pair of forceps and, withdrawing the glass from the flame, pull it out into a filament. Introduce the coloured filament thus obtained into the fringe of a Bunsen flame, and allow the glass, as before, to mould itself into a spherical shape.

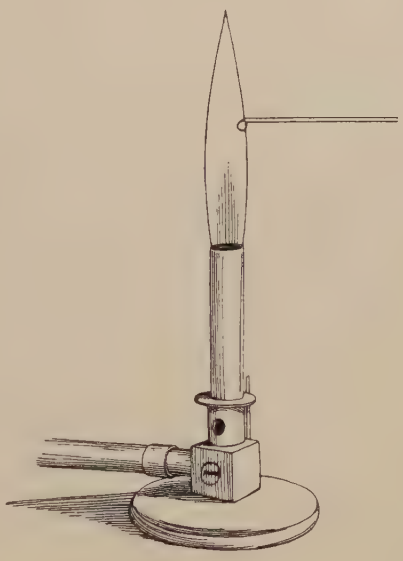


FIG. 2.

SHOWING METHOD OF MAKING A GLASS SPHERULE FROM A GLASS FILAMENT.

3. Arrangements for obtaining the different types of illumination required for the development of the outline and object picture.

The form of illumination which is required for seeing the spherule in the form of a picture in relief is obtained without any special arrangements. There will in each case be patches of light reflected to the eye from the surface of the spherule which faces the window, and in each case there will be an area of comparative shade on the far side from the window.

The varieties of illumination which are required for the development of the other varieties of object pictures can be conveniently

[1] For the purposes of our experiments it will be convenient to employ spherules not less than two millimeters in diameter and to leave in each case a portion of the filament attached in the form of a handle.

obtained by placing the spherules in position against the various *radiant fields*[1] which are furnished on Plate I.

4. Experiments on the production and effacement of outlines.

Experiment 1. Taking hold of an uncoloured glass spherule by its capillary stem, place it in position against a radiant field constituted by a white disc disposed on a black background. (Plate I, Fig. A).

Screen off so far as possible any light which may be incident on the upper surface of the object.

Note that, when the spherule is held at an appropriate distance (say 4–6 inches) from the illuminating field, it will appear as represented in the inset figure as a bright object delineated by a dark outline on a bright background.

Observe also that the breadth of the dark outline diminishes and increases as the spherule is brought nearer to, or, as the case may be, is carried away from, the radiant field.

Experiment 2. Substituting now for the radiant field just employed a bright field enveloping a black disc such as is provided in Plate I, B, and again bringing the spherule into position at a suitable distance from the central disc, observe that the sphere is now, as in the inset figure, delineated by a bright outline upon a black background.

Experiment 3. Substituting again for the radiant field employed in *Experiment* 2 a homogeneous white field such as is provided in Plate I, C, notice that the object is no longer delimited by any conspicuous outline. It will now, apart from reflections from its surface, be almost indistinguishable from the background.

Experiment 4. Substituting for the black and white fields employed in *Experiments* 1–3 a parti-coloured illuminating field consisting of a blue disc mounted centrally on a red field, as provided in Plate I, D, place the object in position at a suitable distance from this, and note that it appears, as shown in the inset figure, as a blue object delineated upon a blue background by a red outline.

Experiment 5. Place the sphere in position against any brightly coloured illuminating field (e.g. a blue field or a red field), hold it some little distance away, and shade off, as you do so, from its surface the colourless light which may otherwise fall upon it, and be reflected from it to the eye. Note that the spherule is now seen as a blue or red element delineated by a dark margin on a field of blue or red (Plate III, Figs. *f* and *g*).

Experiment 6. Now allow a light from a window to fall upon the sphere, and note that the outline, instead of being black, is now, as is the case when Figs. *f* and *g* are viewed through the covering tissue paper, tinted with the colour complementary to the colour of the illuminating field, i.e., as the case may be with yellow and green.

[1] These may be prepared for class instruction purposes by punching out, by means of a cork cutter, discs from white, black, and coloured paper respectively, and mounting these on appropriate backgrounds.

5. Deductions from the above experiments.

We have learnt from the above—

(*a*) That where a spherical object possessing a high refractive index is illuminated from a light source of limited extent placed at some distance behind it on the axis of vision, we have in the retinal image an object delimited by a dark outline upon a bright field.

(*b*) That when the highly refractive object is illuminated from an enveloping radiant field, we have in the retinal image an object delimited by a bright outline upon a dark field.

(*c*) That where the spherule is illuminated both from a light source placed directly behind it and from a light source which radiates light upon it from every side, the retinal field is blank.

Consideration of Fig. 3 below will show that the blank picture obtained in *Experiment* 3 *supra* is a composite of the pictures obtained in *Experiments* 1 and 2.

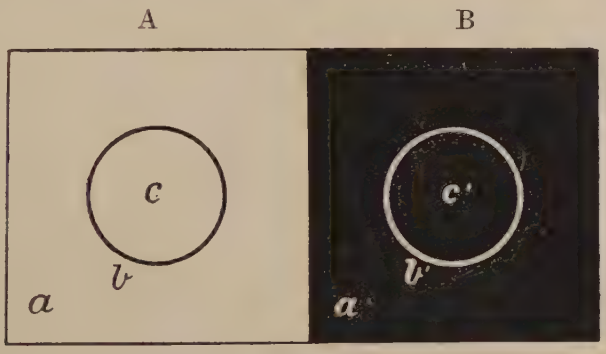


FIG. 3.

SERVING TO ILLUSTRATE THE FACT THAT A BLANK PICTURE IS OBTAINED BY THE IMPOSITION OF A "BRIGHT OUTLINE PICTURE" UPON A "DARK OUTLINE PICTURE."

The superposition of the bright central disc *c* of A upon the dark central disc *c'* of B, gives us the bright central disc in the composite image of A and B.

The bright annulus *b'* in B, superposed upon the dark annulus *b* in A, gives again a bright marginal annulus in the composite image of A and B.

Lastly, the bright field *a* in A, superposed upon the dark field *a'* in B, gives us a bright field in the composite image.

The lessons learnt from the three first experiments are enforced by the lesson of *Experiment* 4. It is here brought clearly before the eye (*a*) that the light which radiates into the pupil from the central area of the sphere and from the surrounding field is light

whose source is to be found on the visual axis directly behind the object, and (*b*) that the light which radiates into the pupil from the marginal area of the sphere is obliquely incident light derived from the outlying regions of the illuminating field.

6. Manner in which outlines are produced and suppressed.

We pass to consider why the radiant points which constitute the object picture arrange themselves in *Experiment* 1 in such a manner as to leave a dark annulus round the margin of the object; in *Experiment* 2 in such a manner as to furnish a bright annulus; in *Experiment* 3 so as to furnish a blank picture, and in *Experiment* 4 in such a manner as to furnish a red outline on a blue field.

We shall most conveniently deal with this problem by conceiving of the light projected upon the spherule as consisting in each case of a system of parallel rays, derived, as the case may be,

FIG. 4.
SHOWING THE MANNER IN WHICH THE "DARK OUTLINE PICTURE" IS PRODUCED.

from a light source disposed immediately behind, or as the case may be excentrically behind, the object.

(*a*) *Formation of dark outlines in the case of a highly refracting air-enveloped spherule illuminated by central white disc disposed on a dark field.*

Figure 4 illustrates the formation of dark outlines in the case

of an air-enveloped spherule upon which is projected a pencil of rays running parallel to the axis of vision.

In the optical section which is here in question four sets of rays lettered *a*, *b*, *c* and *d* respectively come into consideration. The rays *a*, passing as they do wide of the glass sphere, give origin to a system of points *a′ a′*, which radiating into the eye produce the bright object field against which the dark outline of the spherule is defined.

The rays *b* and *c* are in part reflected away from the outer surface of the spherule, in part they pass into the spherule undergoing refraction as they enter. The refracted component of *c* along with the axial ray *d*, which passes through the spherule undeflected, are

FIG. 5.
SHOWING THE MANNER IN WHICH THE "BRIGHT OUTLINE PICTURE" IS PRODUCED.

received into the eye forming the bright central area of the spherule. It will be seen that the gap *a′* to *c′*—*c′* being the point upon the equator upon which the ray *c* is projected from the eye of the observer—corresponds to the dark marginal area which delimits the spherule.

(*b*) *Formation of bright outlines in the case of a highly refracting air-enveloped spherule illuminated by a bright field surrounding a central dark spot.*

Figure 5 illustrates the manner in which bright outlines are produced in the case of a glass sphere, enveloped in air, and illuminated by a corona of obliquely incident light such as is projected upon the sphere when placed in position against a black central disc mounted on a white field.

Again, the incident rays which come into consideration are represented as pencils of parallel rays. The component rays are designated on each side respectively by the letters a', b', c', d, c, b, a.

The rays d and d which pass through the centre of the sphere, and on either side the rays c and c', which traverse the central as distinguished from the peripheral regions of the sphere, fall outside the field of view of an observer who looks down upon the sphere. As a result, the central region of the sphere is dark in the retinal image.

The rays a', a which pass to one side and the other of the sphere, fall in the same manner out of the field of view, leaving the field of the image dark.

There are, however, as the diagram makes clear, other rays which enter the observer's pupil. There is on either side the refracted component of each of the rays b and b. This, as will be seen, is bent up in such a manner as to enter the pupil of the observer. There is further on either side the reflected component of the rays b' and b', which is bent upwards by reflection from the external surface of the sphere.

The refracted and reflected components of b and b', on either side, mutually aiding each other, give origin to the bright outline which delineates the spherule.

(c) *Suppression of the outlines in the case of a highly refracting air-enveloped spherule illuminated by a combination of axial and oblique rays.*

Figure 6 exhibits in a simplified form the conditions we have to deal with when an object is illuminated by a homogeneous illuminating field which we may for our purposes regard as sending out a centrally disposed pencil of axial and parallel rays and two beams of parallel and obliquely incident rays.

The blank image obtained under these conditions is, as has already been explained, the result of the imposition of the image produced by the oblique rays upon the image produced by the axial rays. The manner in which this is achieved will be realized by noting that in the diagram the components of the obliquely incident beams which come into consideration in connexion with the formation of the bright outlines are projected back by the observer into the marginal gap (a' to c') on the equatorial plane, which constitutes in the case where only axial illumination is employed the dark outline which envelopes the spherule.

(*d*) *Development of the red outline on the blue ground in the case where a highly refracting air-enveloped spherule is illuminated from a central blue disc disposed on a red ground.*

Fig. 1, Plate V, which will be understood without verbal gloss, sets forth to the eye the manner in which the red outline and blue field are obtained in the case where the spherule is in position against a parti-coloured radiant field.

7. Influence exerted by the refractive index of the enveloping medium upon the development and suppression of outlines.

Although it will already have been appreciated that the deflection of the incident ray—which is, as we have seen, the essential precondition of the development of outlines—depends upon the immersion of the object in a medium of different refractive

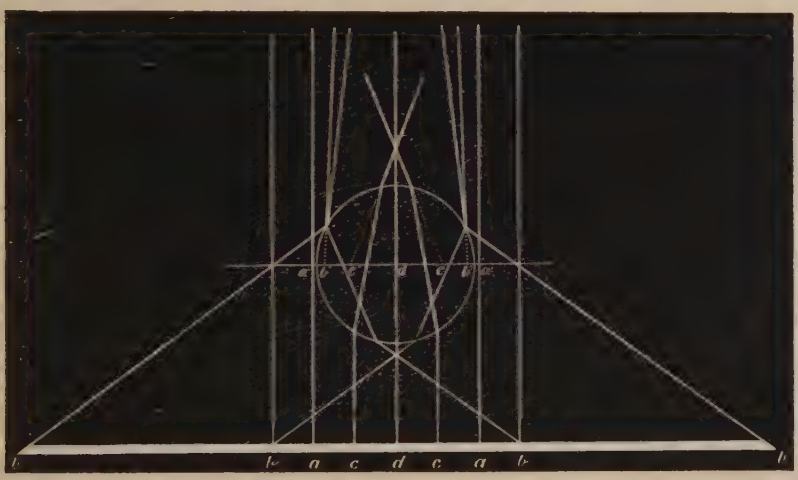


Fig. 6.

SHOWING THE MANNER IN WHICH THE OUTLINES ARE OBLITERATED.

index, it will none the less be useful specifically to draw attention to the influence exerted by the refractive index of the enveloping medium upon the development of outlines. The following experiments may be undertaken :—

Experiment 1. Take a series of flat-bottomed watch-glasses. Keeping one of these empty, fill in the others in series with alcohol, water, 50 per cent. glycerine, and Canada balsam.

Introduce into each watch-glass an uncoloured glass spherule, and employ in each case that form of illumination which is most favourable to the development of a dark, or, as the case may be, of a bright outline.

In other words, employ in the first case a small bright disc, or point source of light, disposed on a dark background, and in the latter case a bright field surrounding a black disc.

Note that as you pass from the enveloping medium of the least refractive index—air—to the enveloping medium of the greatest refractive index—Canada balsam—the outline of the spherule becomes more and more attenuated, until the object is practically invisible even when the conditions of illumination are those most favourable to the development of outlines.

Experiment 2. Hold a watch-glass in position in each case against a parti-coloured illuminating field. Note that when reflection and refraction have been abolished by the mounting of the object in a medium of equal refracting index, it is no longer possible to achieve the delineation of the spherule by a red margin on a field of blue.

Experiment 3. Introduce into each of the four watch-glasses a coloured spherule, and illuminate it as in *Experiment* 1.

Note that we obtain in the first of the series of watch-glasses an outline and colour picture in which the colour element is overborne by the outline, while we obtain in the second and third watch-glasses a progressively purer colour picture, and, finally, in the fourth watch-glass a perfectly pure colour picture.

Experiment 4. View the coloured and uncoloured spherules contained in the three last watch-glasses in each case against a uniform radiant field.

Note that under the combined illumination by axial and oblique rays which is thus achieved, the outlines are in each case attenuated and the colour picture is brought out in greater purity.

Section II.

EXPERIMENTS ON THE DEVELOPMENT OF THE OBJECT PICTURE IN THE CASE WHEN THE OBJECT IS VIEWED WITH THE MICROSCOPE.

1. Introductory.

The conditions, under which the *object picture* or, as we shall henceforth more frequently call it, the *stage picture* is developed in the case of objects which are to be imaged on the retina by the aid of the microscope, are, as has already been intimated, precisely the same as those under which the *object picture* is developed in the case of macroscopic objects imaged on the retina without the aid of any magnifying system.

In setting forth below a series of experiments which make this clear, it will be convenient to assume that the reader has made himself familiar with the method of focussing the sub-stage con-

denser, and of developing in the optical plane occupied by the microscopic object, as the case may be—

(*a*) A system of luminous points radiating light along the optical axis of the microscope, and in each case outwards from that axis through a small angle.

This form of illumination may, without serious inaccuracy, be taken as equivalent to that which would be obtained from a pencil of rays running parallel to the optical axis, such as was postulated in *Cap.* II, *Sect.* I, *subsec.* 6, (*a*), *supra.*

(*b*) A system of luminous points radiating light through a narrow angle along an axis disposed obliquely to the optical axis of the microscope.

Such a form of illumination would be equivalent to illumination by a beam of obliquely incident parallel rays.

(*c*) A system of luminous points radiating light in each case in the form of a hollow cone in all directions at a considerable angle to the optical axis of the beam.

This form of illumination would, in the case of an object encompassed by such radiant points, be equivalent to the illumination by a corona of obliquely incident rays.

(*d*) A system of luminous points radiating light, both along the optical axis of the microscope, and outwards from this through a considerable angle, in such a manner as to form a wide-angled solid cone.

This form of illumination would be equivalent to the combined illumination by pencils of axial and oblique rays.

(*e*) A system of luminous points radiating light of one colour along the optical axis of the beam and outwards at a small angle from this; and light of another colour at a greater angle to the optical axis of the beam in the form of a hollow cone.

This form of illumination would be equivalent to the illumination of the object by such a parti-coloured radiant field as was employed in *Experiment* 4 in *Cap.* II, *Sec.* I, *subsec.* 4.

2. Method of making the apparatus required for the subjoined experiments.

A parti-coloured stop,[1] such as that represented in Plate I, Fig. E, 3, is required for the experiments. Further simple-coloured stops,

[1] Parti-coloured stops, made as described overleaf, have been regularly employed by the author in his practical classes for the last twelve, or more, years for the purposes for which they are employed in this book. Rheinberg, independently taking up and developing the subject of *Illumination by differentially coloured light*, has in his papers (*Journal of the Royal Microscopical Society*, 1896 and 1899 and elsewhere) called attention to many interesting applications of parti-coloured stops.

and in the case where the microscope is not already equipped with these, an annular stop, and a central spot stop (Plate I, Fig. E, 2 and 1) will be required.

(*a*) *Method of making the sub-stage stops.*

Parti-coloured stop.—Soak two pieces of filter paper, the one in a solution of eosine, the other in a solution of methylene blue. After drying them thoroughly, punch out by the aid of appropriate cork-cutters from the blue filter paper a disc about 1 cm. to 1·5 cm. in diameter, and from the red paper a disc about 2·5 cm. in diameter. From the centre of the red disc punch out again a disc of the same diameter as the blue disc. Now mount the red annulus thus obtained on an ordinary microscopic slide in a drop of Canada balsam, fit into it the blue disc, and, taking care that there is plenty of balsam, cover the whole with a cover-glass, keeping the preparation clear of bubbles of air.

Single-coloured stops.—Place a drop of a solution of eosin, methylene blue, or other aniline dye on a microscopic slide and cover it with a cover-glass.

Annular and central spot stops.—These are simple wings and discs cut out of black paper by the aid of cork cutters.

3. Experiments with glass spherules and cylinders.

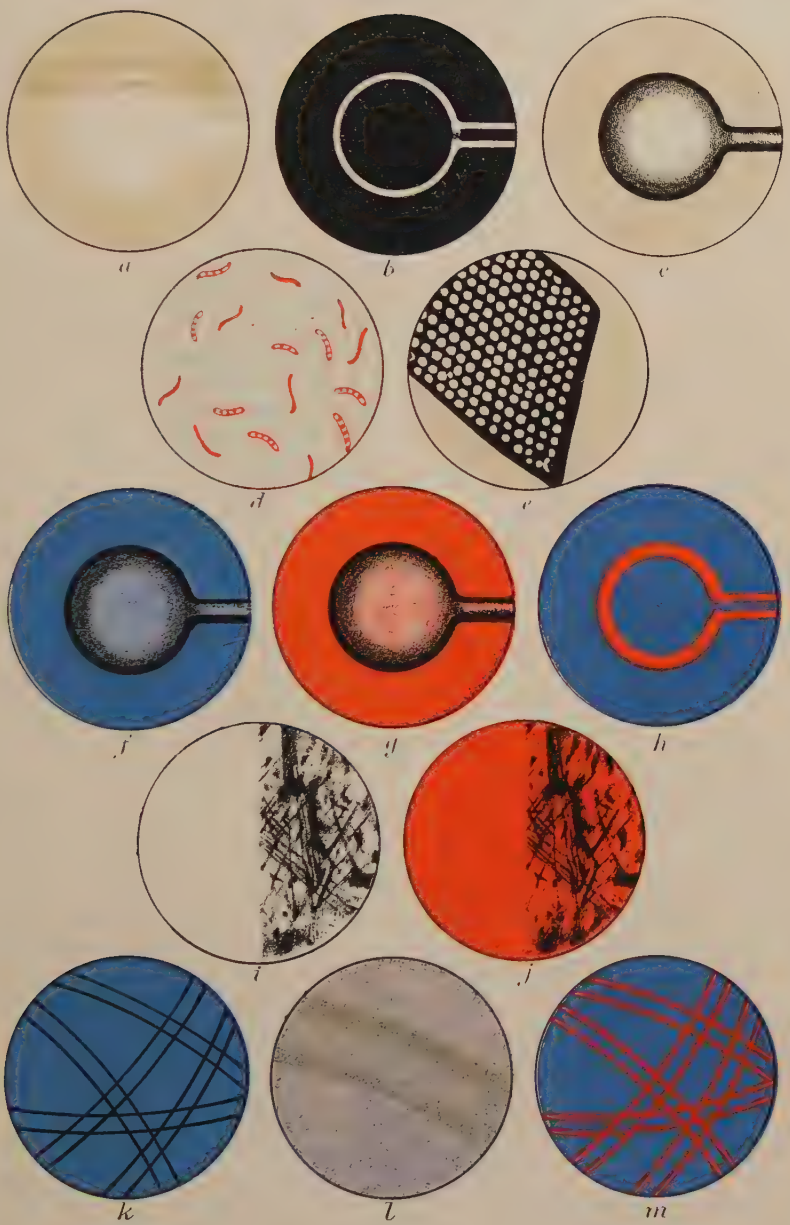
Experiment 1. Mount an uncoloured glass spherule similar but smaller than those in the experiments employed in the preceding section, in a thin watch-glass containing a sufficiency of water to secure complete immersion. Place this watch-glass on the stage of the microscope.

Now fit to the microscope a 1-inch or other low power objective and focus down upon the spherule. Then by the method described in *Cap.* XIII, *subsec.* 4, focus the condenser upon the equatorial plane of the glass spherule. This done place beneath the aperture of the condenser the annular stop represented in Plate I, Fig. E, 2, or, as an alternative, close down the aperture of the iris diaphragm till it transmits only a comparatively narrow cone of light.

Note that the spherule is now, as was the case in *Experiment* 1, *Cap.* II, *Sec.* I, *subsec.* 4, *supra*, outlined by a dark margin. (Plate II, Fig. *c.*)

Rack down the condenser, and note that, as the beams which fall upon the spherule become narrower and narrower by the elimination of the more obliquely incident rays, the outline becomes broader and broader. The conditions will now be precisely analogous to those in the experiment above referred to, where the sphere was carried away to a distance from the white central disc, which functioned as the source of illumination.

Experiment 2. Bring up the condenser again into focus upon the equator of the spherule. Opening wide the iris diaphragm, place centrally under the condenser a black spot stop such as is represented in Plate I, Fig. E, 1. and screen off with the hand the light which is incident on the upper surface of the object. Note that the spherule is now, as in *Experiment* 2, *Cap.* II, *Sec.* I, *subsec.* 4, outlined on a dark field by a bright outline. (Plate II, Fig. *b.*)


a
b
c
d
e
f
g
h
i
j
k
l
m

Rack down the condenser, and note that the bright outline fades out as those components of the beams which emerge from the peripheral zone of the condenser now intersect below the level of the microscope stage and go wide of the spherule (*cf.* Figs. 73 and 90).

Experiment 3. Re-focus the condenser upon the equator of the spherule and remove all stops. Note that the spherule is, as in the case of *Experiment* 3 in *Cap.* II, *Sec.* I, *subsec.* 4, now very faintly outlined. (Plate II, Fig. *a*.)

Experiment 4. Rack down the condenser until its upper face is, say, about an inch below the level of the microscope stage, and place centrally under the condenser the parti-coloured stop whose preparation has just been described. Now screen off with the hand the light which is incident upon the upper surface of the spherule. Note that the spherule is now, as in *Cap.* II, *Sec.* I, *subsec.* 4, *Experiment* 5, seen as a blue object outlined by a dark margin on a field of blue. (Plate II, Fig. *f*.)

The effect in the present case is, as will appear on referring to Plate XII, Fig. 2, due to the circumstance that the blue central element of the beams which issue from the condenser here alone impinges upon the spherule, the red rays from the periphery having intersected below the level of the stage and gone wide.

Experiment 5. Maintaining the coloured stop under the centre of the aperture, re-focus the condenser and again shield off with the hand the light falling on the upper surface of the spherule. Note that the spherule will now appear, as in *Exp.* 4, *Cap.* II, *Sec.* I, *subsec.* 4, as a blue object outlined with red on a field of blue. (Plate II, Fig. *h*.)

The new effect is, as will be clear from examination of Plate V, Fig. 1, due to the fact that the red rays from the peripheral zone of the condenser now impinge upon the focal plane on which the spherule is disposed, and are now, by the refracting and reflecting action of the spherule, carried upwards into the objective.

Experiment 6. Keeping the parti-coloured diaphragm in position, or employing in lieu of this a single blue stop, now make provision for reflection of light into the objective from the upper hemisphere of the spherule. This may be most easily done by bringing the object as it lies on the stage of the microscope either into direct sunlight or into the full light of a lamp.

Now regulate the diaphragm in such a manner as to balance so far as may be practicable the transmitted light against the light reflected from the upper surface of the sphere. As soon as the balance has been established between these, upper and lower, lights, the marginal area of the sphere will assume by subjective contrast a yellow coloration, i.e., a coloration complementary to that of the field.

If the peripheral red portion of the parti-coloured stop, or in lieu of this, a single red stop, be now disposed under the aperture of the substage diaphragm, the field and centre of the spherule will be red and the marginal area will be blueish green.

On shading off the upper light and suddenly readmitting it, the dependence of the coloration of the marginal area upon the reflection

of light from the upper hemisphere of the spherule becomes very manifest to the eye.

It may be noted that the conditions of illumination which are established by the lighting up of the upper surface of the glass spherule in the above experiment are exactly the same as those which are established in connexion with Figs. *f* and *g*, Plate II, when these are seen through the covering layer of tissue paper. When the figures are viewed in this way, it will be found that the dark outlining margin of the sphere will appear as yellow or blueish green respectively, according as it is laid down on a field of blue or, as the case may be, of vermilion.

4. Experiments with blood films.

The above experiments with glass spherules may conveniently be supplemented by experiments with blood films.

Preparation of dried blood films. Blood films can be prepared in the following manner :—

Place a small drop of blood on a microscopic slide which has been roughened by rubbing with very fine emery paper.[1] Bring down upon the drop of blood the edge of another slide, holding the second slide at an acute angle to the first. Wait then till the blood has run about three-fourths of the way across the slip spreading out between the angle on the slides, then draw the upper slip along the surface of the lower one with a steady movement, diminishing the angle between the slides when too thick a film is left behind, increasing the angle when that film is too thin; and allow the film to become air-dry.

Experiment 1. Take such a dried blood-film, place it upon the stage of the microscope, shutting down the iris diaphragm under the condenser, and project upon it in this manner a system of beams of very narrow angular aperture.

Examine the preparation with a low power objective and a high ocular. Note in particular the following points :—

Red blood corpuscles.—These are seen as a combined outline and colour picture, each red blood corpuscle appearing as a pale yellow element outlined with a heavy dark margin. (Plate III, Fig. *a*.)

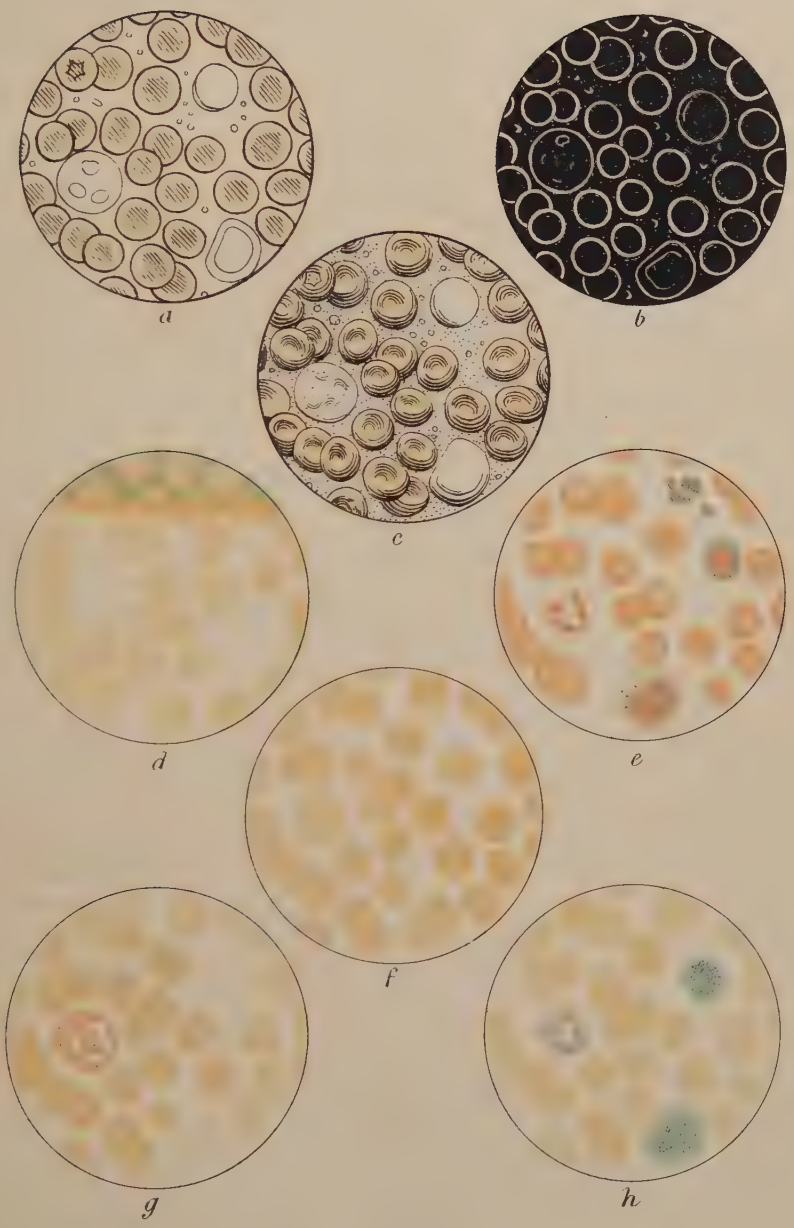
Note that this margin appears by subjective colour contrast somewhat blueish when light is reflected to the eye from its surface.

Internally to this there is developed a bright annulus. The centre of the corpuscle is occupied by a darker area. From these data we may, by a process of inference which we shall hereafter have to consider, arrive at the conclusion that the red blood corpuscle is a highly refracting element possessing the configuration of a bi-concave disc.

White blood corpuscles.—These are seen as colourless elements outlined by margins somewhat less dark than those of the red blood corpuscles. In the interior of the corpuscle there can be discovered on careful focussing another system of outlines marking out, on the hyaline, or, as the case may be, finely granular, background of the corpuscle, one or more highly refracting elements.

[1] The most suitable emery paper is that known in the trade as Hubert OO.

Plate III.



The external outlines here in question correspond to the margin of discs into which the spherical white blood corpuscles have been converted during the making of the film. The internal system of outlines delineates a simple or, as the case may be, a fragmented nucleus. The uniform or finely mottled ground of the corpuscle corresponds to a hyaline or, as the case may be, to a finely granular cellular protoplasm.

Other formed elements.—Brightly refracting objects scattered here and there through the field, furnish indications of the presence of blood platelets.

Experiment 2. Focus the condenser upon the preparation, and then, opening wide the sub-stage diaphragm, place underneath its aperture a central spot stop.

The appearance of the specimen, seen under a low objective and high ocular, will now be as in Plate III, Fig. *b*. Note that nothing can be made out with regard to the configuration in relief of the corpuscle. The stage picture as in Plate II appears perfectly flat.

Experiment 3. Keeping the condenser in focus on the preparation, remove all stops. Note that all the outlines are now effaced, with the result that the white corpuscles are rendered almost invisible, while the red blood corpuscles appear now only as pallid, flattened yellow discs. (Plate III, Fig. *d*.)

Experiment 4. Without altering the vertical height of the condenser, throw it considerably out of centre, as shown in Plate XII, Fig. 1, or alternatively block out all the light except that which is transmitted from a small area on its extreme outer edge.

When an isolated beam of appropriate obliquity has in this manner been projected upon the blood preparation, a picture in relief such as that represented in Plate III, Fig. *c*, will be brought into view, and the heights and hollows of the red blood corpuscles will stand out in stereoscopic relief. The white blood corpuscles will be seen as alabaster-like discs upon which is imprinted, or, as the case may be, moulded as in bas-relief, the form of a nucleus.

5. Comment on the above experiments.

The foregoing examples have furnished us with illustrations of all the different varieties of stage pictures.

Experiment 1 has furnished us, in the case of the hyaline white blood corpuscles, with examples of the pure outline picture, and in the case of the red corpuscles with examples of the combined colour and outline picture.

Experiment 2 has furnished us with an example of *dark ground illumination;* that is, of an outline picture in which objects are delineated by bright outlines on a dark background.

Experiments 4 and 3 have furnished us respectively with examples of the picture in relief, and of the pure colour picture.

The colour picture obtained (Plate III, Fig. *d*) was manifestly defective in many respects. It was defective, in the first place, in the respect that the white blood corpuscles were unrepresented in the picture. It was defective, further, in respect that the coloration of the red blood corpuscles was too pallid to bring out clearly their configuration in plan, or to tell us anything about their configuration in relief.

The defects which have just been pointed out are remediable by staining.

6. Experiments with stained blood films.

One of the best of all methods of staining blood films is Leishman's modification of Romanovsky's method. The staining procedure is carried out as below :—

Method of preparing a stained preparation of blood.

Take a dried blood film and pour over it a few drops of Leishman's staining fluid. After waiting for a few seconds, dilute the stain with somewhat less than an equal volume of water. Allow the diluted stain to remain upon the specimen for two to three minutes, then wash off the dye with distilled water, and allow the distilled water to exert its action for the space of one to two minutes. This done, dry off the water by pressing down a piece of filter paper upon the specimen, and allow the drying process to complete itself without resorting to heat. When perfectly dry, mount the specimen under a cover-glass in Canada balsam, or, dispensing with the cover-glass, in cedar wood oil.

Experiment 1. Place a film stained by the procedure just described upon the stage of the microscope. Illuminate it with a fully open focussed condenser, and bring it into view under an oil immersion lens.

The appearance of the specimen will now be as represented in Plate III, Fig. *e*.

It will be noticed with regard to the red blood corpuscles that for their natural pallid yellow tint there is now substituted a saturated pink. Owing to the greater density of this coloration, there is now a sensible difference in light absorption as between the central area and the margin of the corpuscles. This reveals the fact that the corpuscles are hollowed out in the centre.

In the case of white blood corpuscles, the nucleus which was in the case of the unstained blood films either invisible or only indistinctly visible, now stands out as a densely stained purple element from the cell protoplasm, which is variously coloured according to the type of white blood corpuscles which is being dealt with. In the case of the polynuclear variety of white corpuscle that protoplasm—itself practically colourless —is seen to enclose a number of very fine red stained granules. In the case of a large mononuclear corpuscle, the cell protoplasm is stained a light blue and is doubtfully granulated. In the case of a small mononuclear corpuscle, the rim of protoplasm is stained a saturated blue.

In addition to the red and white blood corpuscles, there are now

brought into view in the preparation also smaller formed elements—the so-called blood platelets. These are, as shown in the figure, stained in a characteristic manner.

Experiment 2. Make the same dispositions as in *Cap.* II, *Sec.* II, *subsec.* 4, *Experiment* 4. Note that the coloured red blood corpuscles again stand out in stereoscopic relief. Note that while the configuration of the red corpuscles is brought out in a very striking manner, the nuclei of the white blood corpuscles have been rendered invisible.

Several important additional points in connexion with the object picture developed in the foregoing experiments may conveniently be taken into consideration at this stage.

7. Influence exerted by the refractive index of the enveloping medium upon the character of the object picture.

It has already been realized in *Cap.* II, *Sec.* I, *Subsec.* 7, in connexion with the experiments undertaken with glass spherules as viewed by the unaided eye, that the breadth of outline depends in each case upon the refractive index of the enveloping medium, and that the complete effacement of the outlines, which is the *sine quâ non* of the attainment of a pure colour picture, can never be completely realized apart from the mounting of the spherules in a medium of equal refractive index. The lessons just referred to may be re-impressed upon the mind by comparing under the microscope uncoloured specimens of blood corpuscles, mounted respectively in air, plasma, and Canada balsam, with coloured specimens of blood mounted in the same enveloping media, and again with glass spherules mounted in the same enveloping media.

Experiment 1. Place ready to hand (*a*) a dried blood film mounted simply in air, (*b*) a moist film made by spreading out a drop of fluid blood under a cover glass, and (*c*) a dried blood film fixed by momentary immersion in a saturated solution of corrosive sublimate, washed, dried, and finally mounted in Canada balsam.

Search these specimens in turn for white blood corpuscles, and note that in the case of the air-mounted specimen these are easily found, while in the plasma-mounted preparation the white blood corpuscles are much less clearly outlined, and are proportionately more difficult to find, the small mononuclear leucocytes in particular being almost invisible. Finally, in the Canada balsam specimen the leucocytes are quite invisible, even in the case where the sub-stage diaphragm is reduced to the dimensions of the pin point.

Experiment 2. Compare an air-mounted stained blood film with a similar film mounted in Canada balsam. Note that, while, in the former case the dark outlines can, even with the most careful adjustment of the illumination and the most careful focussing of the condenser, only with

difficulty be abolished, in the latter case a pure colour picture is obtained without any special adjustment of the illumination. (Plate III, Figs. *d*—*h*.)

Experiment 3. Place upon the stage of the microscope in succession spherules mounted respectively in air, water, 50 per cent. glycerine, and Canada balsam. Note that while in the first case the dark outline occupies a considerable area of the spherule, it has shrunk in the case of the Canada balsam specimen to such extreme tenuity that the presence of the sphere almost escapes observation. (Plate II, Fig. *a*.)

CHAPTER III.

DEVELOPMENT OF THE OBJECT PICTURE IN THE CASE WHERE THE ELEMENTS, WHICH ARE VIEWED BY THE UNAIDED EYE, OR, AS THE CASE MAY BE, BY THE MICROSCOPE, ARE PILED ONE UPON THE OTHER.

Introductory—Experiments on the conditions which determine the transmission of light through a number of superposed objects—Familiar examples of the dependence of opacity upon refraction and reflection of light from translucent elements, and of transparency upon the abolition of such refraction and reflection—On the dependence of opacity in the case of microscopical preparations upon the refracting and reflecting properties of the constituent elements—Deductions from the foregoing experiments—Method by which refraction and surface reflections may be abolished, and transparency secured, in the case where the interstices of a tissue are already filled in with water.

1. Introductory.

Up to the present we have considered only the case of objects disposed upon a single plane. In connexion with all ordinary microscopic preparations, we have to deal with microscopic elements superposed one upon another. When we are dealing with these, the problem arises as to the nature of the disturbance which will result from the interposition of objects which reflect and refract light between the source of the illumination and the object or, as the case may be, between this last and the objective.

2. Experiments on the conditions which determine the transmission of light through a number of superposed objects.

Experiment 1. Take a piece of glass tubing about three centimetres in length and one and a half centimetres in diameter, and cement it down upon an ordinary microscopical slide in such a manner as to make a cylindrical cell. Introduce into the cell thus constructed either (*a*) a number of glass spherules made by the procedure described above, or (*b*) a number of colourless glass beads previously cleaned with acid, so as to render them perfectly transparent, or (*c*) glass wool, or (*d*) powdered

glass. This done, pass a needle under the floor of the cell, and observe that the contents of the cell completely hide it from view, in spite of the fact that in each case these objects have been fashioned out of a transparent substance.

Experiment 2. Fill into the cell glycerine or any fluid of high refractive index. Note that the contents of the cell, after they have been stirred up, or warmed, in such a manner as to get rid of any intercepted air bubbles, become transparent, and that the needle can now be clearly seen through the interposed objects.

The explanation of the opacity of the contents of the cell, in the case where the interstices of the separate elements were filled in with air, and of the transparency which results from the filling in of the interstices with a fluid of high refractive index is—as will come out clearly on comparing Figs. A and B, Plate IV—due to the circumstance that in the first case the rays of light are deflected and turned back by the intervening objects, while in the second case they pass through undeflected.

The following experiments will serve to demonstrate this to the eye :—

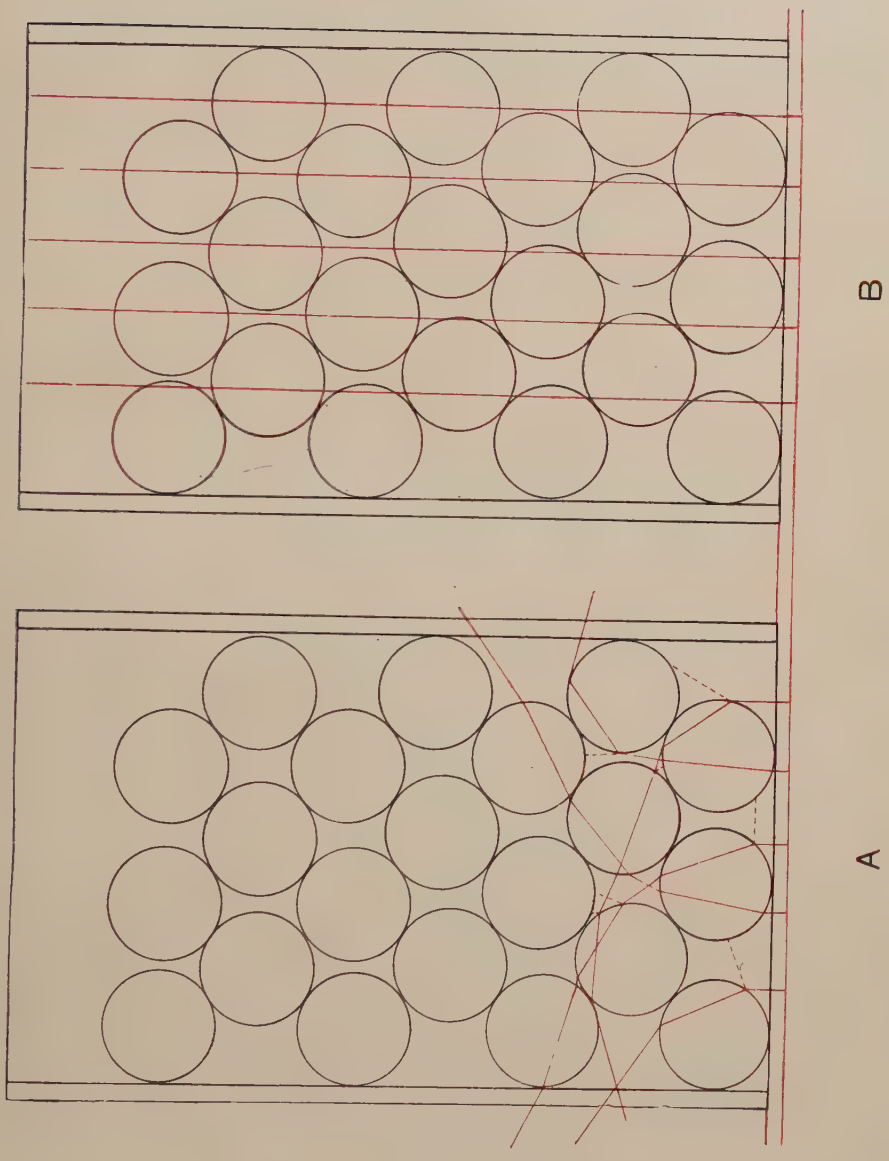
Experiment 3. Take as before a glass cell which has been filled in either with colourless glass spheres, beads, glass wool, or powdered glass. Place the cell either in direct sunlight or in such a position that the light of the lamp falls upon it. Interpose now a sheet of ruby glass between the source of light and the cell. It will then be seen that the red light which is projected upon the surface of the cell, instead of making its way into the depth, is deflected in part outwards through the wall of the cell, and in part directly back in the direction of the source of light.

Experiment 4. Repeat the above experiment with a cell in which the interstices between the glass spheres have been filled in with fluid whose refractive index abolishes refraction and reflection. The red rays will now pass through undeflected, and can be brought into view either by placing a piece of white paper underneath the cell or more simply by holding the cell before the eye and looking through it towards the source of light.

Experiment 5. Fill into a tumbler or beaker a little solution of soap and blow into it through a glass tube until the vessel is filled with bubbles. Cover in now with a piece of coloured glass, and bring the vessel into the full light of the lamp. Note that the coloured light fails to penetrate the froth, and that it is thrown backwards and outwards by reflection and refraction.

3. Familiar examples of the dependence of opacity upon refraction and reflection of light from translucent elements, and of transparency upon the abolition of such refraction and reflection.

PLATE IV.



Falling snow as seen by an observer looking upwards appears dark. This is due to the circumstance that the light which is incident upon the upper surface of the snowflakes is from these reflected back to the sky.

When the relative positions of the observer and the snow crystals are reversed, and the interstices are, as before, occupied by air, the snow is, by reason of reflection, of an opaque white.

As snow thaws, and reflections are abolished, by the entrance of water into the interstices between the flakes, snow loses its whiteness and opacity.

4. On the dependence of opacity in the case of microscopical preparations upon the refracting and reflecting properties of the constituent elements.

What holds true in the case of the glass spheres, the soap bubbles, and the snow crystals must, as consideration will show, hold true also of sections and other microscopic preparations, where we are dealing with highly refracting elements superposed one upon another.

For the purposes of demonstrating that this is actually so, we may experiment with ordinary microscopic sections or, more simply, with strips of filter, or blotting, paper.

Experiment 1. Mount a strip of white filter paper on an ordinary microscopic slide, and place this in position upon the stage of the microscope. Focus down upon it with a one-inch lens, and then, without screening off the light which impinges upon the paper from above, illuminate from below with coloured light obtained by placing the coloured stop or a piece of coloured glass under the fully open condenser. The paper viewed under these circumstances will appear white and practically opaque; only here and there will the coloured light come through to the eye of the observer. (Plate II, Fig. *j on the right.*)

Experiment 2. Repeat the experiment, using, instead of the unstained filter paper, a piece of the paper which has been stained in methylene blue and dried. Use in conjunction with this a piece of red glass in the sub-stage.

Experiment 3. Take in hand now the strip of white paper, turn your back to the window, and hold the coloured glass in such a manner as to throw coloured light on the nearer side of the paper. Note that coloured light is now thrown back to your eye from the paper.

When the strip of paper was on the stage of the microscope, the coloured light was in like manner reflected back from the under surface of the paper, while the colourless light which fell upon the upper surface of the paper was reflected back towards the objective.

Experiment 4. Returning the strip of white paper to the stage of

the microscope, place a drop of cedar oil or of Canada balsam upon it, and illuminate from below with coloured light.

It will be plain now even to naked eye inspection that the coloured light passes freely through the paper in the region of the oil spot.

Examine the preparation by the aid of the microscope, and observe that now the coloured light floods through every pore of the paper over the area occupied by the oil spot. (Plate II, Fig. *j on the left.*)

Take note also in passing of the fact that under the influence of the colourless light which falls upon and is reflected to the eye from the more opaque strands of the paper, these stand out in beautiful colour contrast to the ground tone furnished by the transmitted light. (Plate II, Fig. *j seen through the covering tissue paper.*) The brilliancy of the colour contrast will, in conformity with a general law, be greatest where the illumination of the coloured and uncoloured areas of the preparation is exactly balanced.

Experiment 5. Repeat the above experiment, employing, as in *Experiment* 2, blue paper and a red glass in the sub-stage. Note that now the paper everywhere transmits red light to the eye.[1]

Experiment 6. Take in hand again the strip of white filter paper with the oil spot. Replacing it upon the stage of the microscope, cut off the light from below, and illuminate from above with coloured light. Note that the oil spot will now appear uncoloured, while the surrounding paper will reflect coloured light to the eye. The transparency which is achieved by filling in the interstices of the preparation with a highly refracting medium is thus seen to go hand in hand with loss of reflecting power.

Experiment 7. Place side by side under the microscope a dry strip of filter paper and a strip of filter paper which is impregnated with oil. Closing down the iris diaphragm, screen off the top light and examine the fibres in the region where the interstices are occupied by oil, and again in the region where the interstices are occupied by air. Notice that the contours of the fibres in the former case are, as shown in the figure (Plate II, Figs. *i* and *j on the left*), delicately outlined, and that they are in the latter case broad and dark (Figs. *i* and *j on the right*).

The gain in transparency is thus seen to be associated with a loss in refracting power on the part of the fibres.

5. Deductions from the foregoing experiments.

We learn from these experiments that transparency is achieved only at the expense of those optical differences which are essential to the development of an object picture of an uncoloured element. The necessary corollary of this is, as we shall see, the abandonment of the outline picture and the resort to a pure colour picture in every

[1] This quality of transparency which is conferred upon filter paper by mounting it in a medium of high refractive index was, as the reader will now appreciate, turned to practical account in *Cap.* II, *Sect.* 2, *subsec.* 2, in connexion with the making of the parti-coloured stop employed there

case where—as in the ordinary section—we are concerned to bring into view microscopic elements which are piled one upon the other.

6. Method by which refraction and surface reflections may be abolished, and transparency secured, in the case where the interstices of a tissue are already filled in with water.

When the opacity of a microscopical specimen is due to the fact that the interstices in the preparation are filled in with air, transparency can, as we have seen, be readily secured by directly displacing the air in these interstices by a medium of high refractive index, such as Canada balsam or cedar oil.

The process of *clearing*, or rendering transparent, a microscopical preparation is more complicated when the interstices are filled in with water instead of air. This is due to the fact that the highly refracting oils and resins ordinarily employed for mounting microscopical specimens form in each case a turbid mixture with water. The removal of the water is thus a necessary preliminary to the filling in of the interstices by oil, or, as the case may be, Canada balsam.

The removal of the water may be carried out in two ways. In the case of film specimens, it may simply be driven off by heat. The air which takes the place of the water driven off from the specimen can then, in its turn, be displaced by Canada balsam.

In the case of sections, inasmuch as here mechanical injury might be done to the specimen by the generation of steam in its interior, the contained water must be extracted by the more complicated procedure described in *Experiment* 3 below. The following experiments will be instructive :—

Experiment 1. Take a strip of filter paper and immerse it in water, mount it on a glass slide, and now drive off the water by gentle heating over a flame. Note that the specimen loses transparency as it dries. When thoroughly dried, mount this specimen in Canada balsam. Test its transparency by superposing it upon printed matter or by passing a needle below the slide. Further test its transparency by placing it upon the stage of the microscope, and by employing a coloured stop in the sub-stage, as was done in the experiments in *subsec.* 4, *supra.*

Experiment 2. Place side by side on the glass slide, stirring up together in each case :

(*a*) A drop of water and a drop of Canada balsam ;
(*b*) A drop of alcohol and a drop of Canada balsam ;
(*c*) A drop of oil of cloves and a drop of water ;
(*d*) A drop of alcohol and a drop of water ;
(*e*) A drop of oil of cloves and a drop of alcohol ;
(*f*) A drop of oil of cloves and a drop of Canada balsam.

Note for use in the next experiment that in the first three cases a turbid mixture is produced, and in the last three a transparent mixture is obtained.

Experiment 3. Take a strip of filter paper as in *Experiment* 1, dip it in water, and mount it on a glass slide. Holding the slide on the slant, pour absolute alcohol over the specimen, pouring on the alcohol on the upper end of the slide, and allowing it to run down to, and drip off from, the lower end. Do this repeatedly, testing the successive washings as they collect on the lower edge of the slide, by adding to them in each case a drop of oil of cloves. Note that a milky appearance is produced so long as the washings contain water. Note also that the specimen loses in transparency when the water has been displaced by alcohol.

When the last trace of the water has been removed, flush the specimen several times in succession with oil of cloves until the washings cease to give a precipitate when tested with Canada balsam. Note that the specimen gains in transparency by the displacement of the alcohol by oil of cloves.

When the last trace of alcohol has been removed, displace the oil of cloves in the interstices of the specimen by Canada balsam, allowing the washings as before to run down to the lower end of the slide.

Test the specimen by the methods employed in connexion with *Experiment* 1, and note that, from the point of view of the transparency achieved, the specimen here prepared has no advantage whatever over the specimen prepared by the procedure described in connexion with *Experiment* 1.

The comparison here instituted will serve to correct the common error which attributes the transparency of the Canada balsam specimen to a special " clearing " effect exerted by the oil of cloves. Speaking of the oil of cloves as " clearing " instead of as an " alcohol displacing " agent is, as consideration will show, to insist upon the quite unessential fact that oil of cloves possesses a high refractive index, and to leave out of sight the really essential fact that oil of cloves is, by reason of its compatibility with alcohol on the one hand and with Canada balsam on the other, a suitable intermediary between the alcohol which dehydrates and the final mounting medium which is employed for the clarification and preservation of the specimen.

CHAPTER IV.

ON THE PRINCIPLES WHICH GOVERN THE STAINING OPERATIONS WHICH ARE UNDERTAKEN FOR THE PURPOSE OF DEVELOPING A MICROSCOPIC COLOUR-PICTURE.

Introductory—Fundamental principles of staining—Chemical properties, classification and solubilities of the anilin dyes—Elementary conceptions with regard to the chemical properties of the histological elements of the animal body and their interaction with anilin dyes—Illustrative experiments—Importance of methods of differential staining considered as methods by which microscopic elements can be brought into view and identified.

1. Introductory.

The superiority of the colour picture over every other kind of stage picture and its pre-eminent importance in connexion with the microscope having been made clear in *Caps.* I and III, the reader will already have discerned that the art of staining and the theoretical principles of that art are of pre-eminent importance to the microscopist.

It is not proposed in the present treatise, where we have in view the microscope rather than the microscopic object, to deal in any adequate way with these subject matters. In the following the attention of the reader will be directed only to the fundamental principles which underlie all staining processes.[1]

2. Fundamental principles of staining.

Every staining process depends upon a combination between the chemical substance which constitutes the dye and a particular tissue element which has an affinity for that dye.

The nature of that affinity has not yet been satisfactorily elucidated. While it would seem that something other, or something more, than an ordinary chemical reaction is in each case involved,

[1] For further information the reader may be referred in particular to Gustav Mann's masterly and suggestive *Treatise on Physiological Histology.*

we can in many cases express the events in a staining process in the language of chemical equations. This holds true in particular in connexion with the staining of animal tissues by anilin dyes.

3. Chemical properties, classification and solubilities of the anilin dyes.

Anilin dyes may be regarded as salts containing a colouring element or *chromophor*, united to a base or acid, according as the chromophor in question possesses, in the particular case, acid or basic properties. In the case where the chromophor functions as an acid the dye is denoted an *acid dye*. In the case where the chromophor functions as a base the dye is denoted a *basic dye*.

In the subjoined experiments eosin and methylene blue will serve respectively as examples of acid and basic dyes.

The former may, in connexion with the chemical equations which are hereafter employed to suggest the nature of the chemical reactions involved, conveniently be spoken of as *eosinate of soda*. The latter we may speak of as *hydrochlorate of methylene blue*.

The chromophoric components of these dyes may, in connexion with the equations above referred to, respectively be designated *eosinic acid* and *methylene blue*.

With regard to the solubility of the anilin dyes and their chromophors, all that it will be necessary to say here is, with respect to the first, that they are for the most part soluble in water, and very soluble in alcohol and glycerine; and, with respect to the second, that these are for the most part insoluble in water.

4. Elementary conceptions with regard to the chemical properties of the histological elements of the animal body and their interaction with anilin dyes.

The chemistry of microscopical staining takes its origin in a series of papers on the staining properties of the white corpuscle, which were published by Ehrlich under the title of *Farbanalytische Studien*. The chemical constitution and solubilities of the anilin dyes as set forth above are, in the papers above referred to, for the first time made to furnish an explanation of the events which occur in connexion with staining of white corpuscles.

It is shown, on the one hand, that the histological elements of the body may, according as they combine respectively with basic or acid chromophors, be classified as acid, or *basophil*, and basic, or *oxyphil*, elements. And it is shown that in each case there are degrees and gradations as between the different tissue-elements in the matter of their affinity to acid or, as the case may be, basic stains.

In the case of the histological elements of the blood, the haemoglobin of the red corpuscles and the granules in polynuclear and eosinophilous leucocytes furnish examples of oxyphil elements. The nuclei of the leucocytes, probably by reason of their content in nucleinic acid, are basophil elements.

It is further brought out clearly in the papers above referred to that the combination of the chromophor with the tissue element presupposes not only an affinity as between the chromophor and the tissue element, but an affinity which shall be sufficiently powerful to overcome the bond which binds the dye-stuff to the menstruum in which it is dissolved.

It will be shown, in connexion with the experiments below, that the first of these principles furnishes us with an explanation of the *elective affinity* of different tissue elements for different stains, and also with an explanation of accelerating or, as the case may be, retarding and decolourizing action which is of acids and alkalies respectively.

Similarly, it will be shown in the experiments which follow, that the second principle furnishes an explanation of the fact that tissue-elements are more or less quickly and intensely stained, according as the menstruum in which the dye-stuff is dissolved is capable of holding more or less of that dye-stuff in solution. We shall see that the dilution of an alcoholic solution of an anilin dye by water, and the addition to a watery solution of an anilin dye of such amounts of acid or alkali as will tend to displace the chromophor from its combination and render it available in a state of *suspended precipitation* will in each case be favourable to the combination of the chromophor with the tissue-element.

5. Illustrative experiments.

Experiment 1. Take a watery solution of eosin and add to it acetic acid. The colouring matter is thrown down in the form of a precipitate. Pour off the colourless fluid and treat the coloured precipitate with dilute caustic soda. The dye-stuff is reconstituted and passes into solution.

The changes that take place may be represented as follows :—

(*a*) Eosinate of soda + acid = neutral soda salt + eosinic acid (dissociated chromophor insoluble in water).

(*b*) Eosinic acid (dissociated chromophor) + caustic soda = eosinate of soda (reconstituted dye-stuff, soluble in water).

Experiment 2. Take a watery solution of methylene blue and add to it caustic soda in excess. The chromophor is thrown out of solution.

Experiment 3. Take a watery solution of methylene blue and add to it drop by drop a solution of eosin. A dark granular precipitate which consists of compound of the chromophors of the two dye-stuffs is thrown down (*vide* further *Experiment* 10).

Eosinate of soda + hydrochlorate of methylene blue = eosinate of methylene blue + neutral soda salt.

Experiment 4. Having made a blood film and having fixed it by a momentary immersion in a saturated solution of corrosive sublimate, immerse it, after thorough washing in a one-per-cent. watery solution of eosin. Examine the film now from time to time, washing off the stain in each case under a tap and scrutinizing in particular the end of the film where the white corpuscles are aggregated. It will be noted that first the oxyphil granules in the leucocytes and then the red corpuscles and the protoplasm of the leucocytes take up the stain.

The changes which are associated with the staining of the red corpuscles may be represented thus :—

Eosinate of soda + red corpuscles = eosinated red corpuscles + free soda.

Experiment 5. Take a blood film which has been stained diffusely in eosin, introduce it into a very weak solution of an alkaline salt, e.g. into a 1-in-5000 sodium carbonate solution. Note that the stain is loosened from its combinations and now washes out in water. The decolourizing effect is seen first in the less oxyphil elements. The colour is first discharged from the protoplasm of the leucocyte, afterwards from the red corpuscles, and from the oxyphil granules of the leucocyte.

The changes, so far as they refer to the red blood corpuscles, may be represented as follows :—

Eosinated red blood corpuscles + free soda = red blood corpuscles (decolourized) + eosinate of soda (reconstituted dye-stuff).

Experiment 6. Add to a watery solution of eosin a quantum of acid which falls far short of producing a precipitation in the staining fluid. More intense, and at the same time more diffuse staining is now obtained than is the case with a simple watery solution of the same strength. The effect obtained may be attributed (*a*) in part to the circumstance that the chromophor is here available in a state of suspended precipitation; (*b*) in part to the circumstance that we have here in the staining fluid free acid in sufficient quantity to take up the alkali, which is, as shown above in *Experiment* 4, dissociated from the eosin in the process of staining.

In lieu of the equation given in connexion with that experiment, we have here the equation :—

Eosinate of soda + red corpuscles + free acid = eosinated red blood corpuscles + neutral soda salt.

Experiment 7. Take either a new blood film, or a film which

has been over-stained in eosin and then partially decolourized, and place this film in a one-per-cent. watery solution of methylene blue. It will be seen on washing off the excess of stain in water, that the nuclei of the white blood corpuscles have combined with the basic chromophor.

Nuclei (containing nucleinic acid) + hydrochlorate of methylene blue = nucleate of methylene blue + free acid.

Experiment 8. Take two films of blood which have been stained with methylene blue. Immerse one of these in a weak acid solution and the other in alcohol. In both cases the colour will be discharged.

The changes which are associated with the discharge of the colour from the nuclei by the agency of hydrochloric acid may be represented as follows :—

Nuclei stained with methylene blue + hydrochloric acid = hydrochlorate of methylene blue (reconstituted dye-stuff) + decolourized nuclei.

In the case of decolourization by alcohol, it is a question of the affinity of the menstruum for the colouring matter being greater than that of this colouring matter for the tissue element.

Experiment 9. Having prepared a one-per-cent. solution of methylene blue, in a 1-in-10000 watery solution of caustic soda, immerse in this a blood film previously fixed and washed. Already after the lapse of a second or two the nuclei will be found intensely stained, and, in addition, the protoplasm of the leucocytes and the red corpuscles will have taken on the stain.

The intensifying and accelerating effect of the caustic soda addition is explicable on exactly the same principles as the effect of the addition of acid to an acid dye (*Experiment* 6, *supra*).

The effect so far as it consists in the taking up of the acid liberated from the hydrochlorate of methylene blue in the course of the staining is exhibited in the equation below :—

Nuclei of leucocytes (containing nucleinic acid) + hydrochlorate of methylene blue + free alkali = nucleinate of methylene blue + neutral salt.

Experiment 10. Add, as in *Experiment* 3, a watery solution of eosin to a watery solution of methylene blue, collect the precipitate, which consists, as we have seen, of eosinate of methylene blue, on a filter and dissolve it in methyl alcohol. This constitutes Jenner's stain. Introduce a blood film into the stain and leave it there for three to five minutes.

On examining the film, it will now be found that the oxyphil elements have taken up the colouring matter of the eosin, and the basophil elements the colouring matter of the methylene blue.

These changes in connexion with the red corpuscles and the nuclei can be expressed in the following equation :—

Eosinate of methylene blue + nuclei (basophil) + red blood corpuscles (oxyphil) = eosinated red blood corpuscles + nucleinate of methylene blue.

On Plate III are exhibited in Fig. *f* a blood film overstained with eosin by the method employed in *Experiment* 6; in Fig. *g* the same blood film decolourized by the method employed in *Experiment* 5; in Fig. *h* the same blood film afterwards stained by a solution of methylene blue in a five-per-cent. solution of carbolic acid.

6. Importance of methods of differential staining considered as methods by which microscopic elements can be brought into view and identified.

The fact that particular dyes are fast only upon particular textures—a fact which sooner or later enforces itself upon the attention of every purchaser of hosiery—supplies illustration of one of the practical consequences which follow from the elective affinity of certain dyes for certain elements. A further practical illustration of the operation of the same law is furnished by the fact that it is possible to produce a pattern of colours in a textile fabric by weaving together two different materials and then dyeing the whole in two colours, of which the one is fast on the one material, and the other on the other.

Here the fact that the two elements of the fabric take up each its particular dye is in reality a testimony to the character of those materials.

This method of disclosing and identifying elements which would otherwise remain unidentified is known as *differential staining*. The method is, as will be recognized, that employed in *Experiment* 10 above. It will gradually be borne in upon us as we proceed that microscopical discovery depends—more than upon any other factor—upon the invention and exploitation of new methods of differential staining.

CHAPTER V.

ON THE RESTRICTIONS WHICH HAMPER THE MICROSCOPIST IN THE MATTER OF THE SELECTION AND DEVELOPMENT OF THE STAGE PICTURE; ON THE DEFECTS AND SOURCES OF FALLACY WHICH ATTACH TO THE MICROSCOPIC OUTLINE PICTURE AND MICROSCOPIC COLOUR PICTURE RESPECTIVELY; AND ON THE LIMITATIONS WHICH ARE IMPOSED UPON MICROSCOPIC ACHIEVEMENT BY THE INADEQUATE REPRESENTATION OF THE OBJECT IN THE STAGE PICTURE.

Introductory—On the restrictions which hamper the microscopist in the matter of the selection and development of his stage picture—On the disadvantages and imperfections and fallacies which attach to the microscopic outline picture—On the disadvantages and imperfections and fallacies which attach to the microscopic colour picture—General question of the limitations which are imposed upon microscopic achievement; and appraisement of the relative importance of each of the limitations which come into consideration.

1. Introductory.

By the time he has arrived at the present Chapter the reader will have convinced himself of the truth of the general principle that no object is imaged by the microscope unless the microscopist has first, by the exercise of his art, depicted it in the stage picture. He will also have learned the different procedures by which the object can be depicted.

He may here profitably pass on to certain general considerations which have reference (*a*) to the restrictions which are imposed upon the microscopist in the matter of the choice of his stage picture; (*b*) to the deficiencies of that stage picture according as it is in the particular case an outline or a colour picture; and (*c*) to the limitations which are imposed upon microscopical achievement by the inadequate representation of objects in the stage picture.

2. On the restrictions which hamper the microscopist in the matter of the selection and development of his stage picture.

What is of importance to keep in view in connexion with this subject matter may be compressed into the following series of propositions :—

(1) An outline picture can be achieved only in the case where microscopic objects are disposed upon a single optical plane, and where they differ in refractive index from the medium in which they are immersed.

(2) Even when these conditions are realized, a bright outline picture can, as we shall see hereafter (*Cap.* XIV.), be achieved only when the numerical aperture of the beams, which are brought to focus by the condenser, stands in a certain relation to the numerical aperture of the objective.

(3) In the case where microscopic objects cannot be mounted in a medium of different refractive index, and in the case where they are piled one upon another, the microscopist is limited to the development of a colour picture.

(4) A colour picture can be achieved only in the case where dyes are available which have with respect to the microscopic objects which are to be depicted elective chemical affinities.

(5) Where a preparation is mounted and illuminated in such a manner as to display the outline picture to best advantage, the coloured elements in the preparation will be obscured ; and, *vice versâ*, where a preparation is so mounted and illuminated as to display the coloured elements to best advantage, the uncoloured elements will have been rendered invisible.

3. On the disadvantages and imperfections and fallacies which attach to the microscopic outline picture.

The following are the more important disadvantages which attach to the microscopic outline picture.

(a) *Very minute microscopic elements cannot be brought into view in a satisfactory manner by the aid of outlines.*

The question of the comparative advantages of the outline and colour picture from the point of view of the bringing into view of objects which subtend only a very small angle at the retina has already been considered in *Cap.* I, *subsect.* 4.

It will be remembered that while a coloured element comes into view as soon as its diameter subtends the necessary angle at the retina, an object which is delineated by an outline does not come

into view until the diameter of its delineating outline subtends that necessary angle.

It follows that very minute microscopic elements cannot be satisfactorily brought in view in the outline picture.

(b) *Absence of a definitive focal plane, possibility of misprision of focus, and difficulties in connexion with the interpretation of the picture.*

We have seen that the development of outlines in the case of the microscopic outline picture depends upon the fact that light is turned aside from its course when it impinges upon any element in the preparation which differs in refractive index from the medium in which it is embedded. As rays thus diverted emerge from the microscopic object, their paths must manifestly intersect, as they pass upwards, with the paths of other emergent rays. As a result, we have developed upon a series of optical planes disposed superficially to the microscopic preparation in each case a system of radiant points, corresponding to the nodal points of the intersecting rays. These arrange themselves in such a manner as to form a definite pattern.

The disadvantages which are associated with this are the following :—

(α) The observer may, by mistaking one of these derivative patterns for the fundamental pattern which is positioned in the actual object, lapse into the fallacy of *misprision of focus.*

Illustration of this possibility, which has already been furnished in *Cap.* I, *subsect.* 7, *Experiments* 1 and 2, is further furnished by *Experiments* 4 and 5 below. Let it be observed that in the case of the colour picture, we are safeguarded against the fallacy of misprision of focus by the fact that the derivative pictures are by their diffuseness and inferior definition readily discriminated from the fundamental picture.

(β) Even where the mind of the observer does not mistake a derivative pattern for a fundamental pattern, it is disturbed and perplexed by the development under the microscope at each different level of a different object picture.

It may be noted in this connexion that the dark outline picture which is depicted in Plate III, Fig. *a*, is one arbitrarily selected, out of a whole succession of equally well defined pictures encountered by the microscopic observer as he focusses up and down upon an air-mounted red blood corpuscle. Actual illustration of perplexed situation with which we have to deal in such a case is furnished by *Experiments* 2 and 3 below.

(γ) A conception of the configuration in relief of the microscopic object can be arrived at only by a process of induction by which we follow back, so far as may be, the derivative pictures step by step to their original.

A measure of the difficulty of building up, by an effort of the intellect, out of a succession of fundamental and phantom pictures an adequate conception of the configuration in relief of a microscopic object, will be furnished by the sense of intellectual contentment with which we turn from the consideration of the problem furnished by the sequence of object pictures in *Experiment* 2 below, to the solution of that problem which is supplied by the stereoscopic picture of the red corpuscle (Plate III, Fig. *c*), which is developed under oblique illumination.

Experiment 1. Place upon the stage of the microscope under a fully open condenser a system of opaque and transparent rulings (the system of rulings which is provided in the pocket of the cover will serve in default of a finer system). Bring these into view under a low-power objective, and then focus up until the fundamental picture has disappeared and there has appeared in its place a phantom picture of bright and dark bands, produced by the intersection of the beams which take origin in the clear lines of the ruling.

Comment. While this experiment furnishes illustration of the production of a phantom pattern, it is not intended to illustrate the possibility of confusion through misprision of focus. The fact that the phantom pattern is here wanting both in sharpness and detail sufficiently safeguards us in this instance against confusing it with the fundamental pattern.

Experiment 2. Place upon the stage of the microscope an unstained air-mounted blood film. Focus down upon a red corpuscle with a high-power dry objective.

Note that we cannot, as we focus, pitch upon anything in the nature of a definitive focal plane. We encounter instead, through a certain depth of focus, a succession of different and in each case sharply defined images. According as we focus up or down, the central area of the corpuscle comes out, as the case may be, as dark or bright; the annular ridge of the corpuscle comes out, as the case may be, broad or narrow; and the delimiting margin of the corpuscle appears, as the case may be, dark or bright.

Comment. Figs. 2 and 3 on Plate V will convey some sort of an idea of the optical situation with which we are dealing in the case of a dark outline picture and a colour picture respectively.

In Fig. 2, Plate V, where we are dealing with the conditions which obtain where a coloured red-blood corpuscle is viewed in a medium of lower refractive index, the beams which pass through the object are opened out or, as the case may be, closed up by refraction in such a manner as to form, by the intersection of their component rays with those from the field, upon a series of optical planes superficial to the object, in each case a different pattern of radiant points.

According as we focus upon the optical plane A or the overlying

PLATE V.

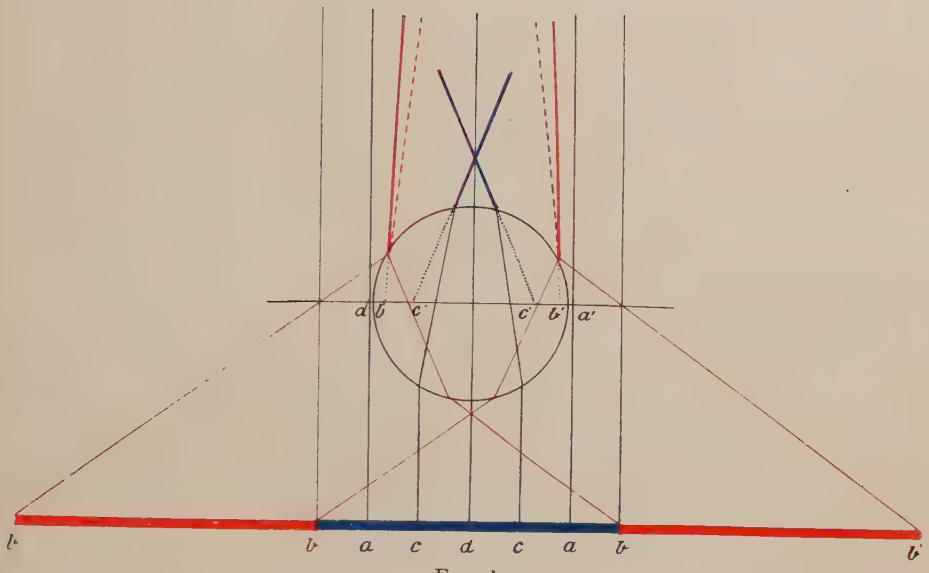


FIG. 1.

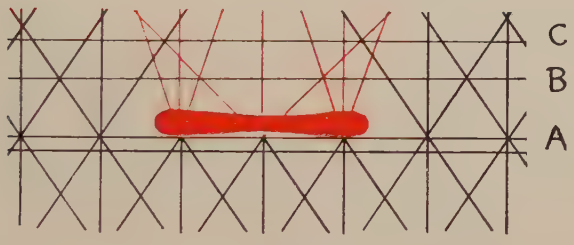


FIG. 2.

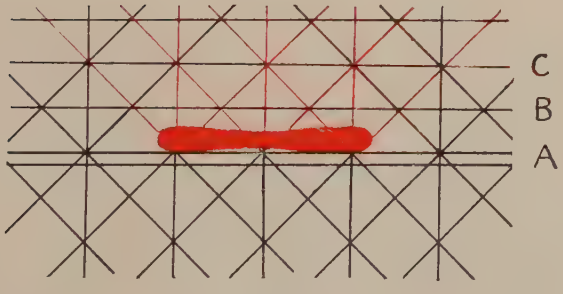


FIG. 3.

optical planes B and C, the object picture under observation will, as the case may be, be the pattern of radiant points and dark points which is positioned in the object itself, or one of the derivative patterns formed by the intersection of the rays which proceed from the object with the rays which proceed from the field.

Turning to Fig. 3, Plate V, which represents the conditions which obtain where a coloured red-blood corpuscle is mounted in a medium of equivalent refractive index, we see that as we focus up from the plane upon which the red corpuscle is disposed, we encounter derivative pictures which are progressively more and more diffuse. The diagram makes clear that this progressive diffuseness depends upon a progressively increasing dilution of the red light from the corpuscle with the colourless light from the field.

Experiment 3. Seek out now in the blood film a coarsely granular leucocyte. Note the changes from dark to bright and bright to dark which occur in the object picture with every movement of the fine adjustment.

These changes are conditioned by the shifting of the focus from the fundamental to the phantom picture and back again from the phantom picture to the fundamental picture.

Experiment 4. Place a drop of blood on the slide under a cover glass, and allow evaporation to proceed until the plasma becomes somewhat inspissated. Water will now pass out into the plasma from the interior of the red blood corpuscles, and the latter will be distorted into prickly spherical elements.

The surface inequalities of these elements will now deflect the course of the transmitted light in all directions, with the result that there will come into existence a meshwork of interlacing rays whose nodal points can only with difficulty be distinguished from the radiant points which constitute the underlying fundamental object picture.

Experiment 5. Place one of the air-mounted diatoms, which are commonly employed in connexion with the testing of objectives, on the stage of the microscope under an oil immersion lens.

It will, here again, be noted that a process of inversion from dark to bright and vice versâ takes place in sympathy with every up and down movement of the focus. It will further be observed that in the case where the surface of the diatom slopes away towards its edge, the derivative and the fundamental object picture are in view simultaneously in different parts of the specimen.

Comment. It will be unnecessary, in view of what has been said in connexion with β and γ, *supra*, to enforce upon the reader's attention the consideration that the great uncertainty which prevails as to the real configuration of various diatoms is in large measure the result of the difficulty of distinguishing the fundamental from the derivative pattern, and of deducing the former from the latter.

(c) *Elements, in the object which are dark by reason of the deflection of light, may be confused with elements which are dark by reason of the presence of pigment.*

Dark points such as those which are encountered in the crenated corpuscle (*Experiment* 4, *supra*) at a particular adjustment of the focus are, as will be obvious to reflection, capable of being confused with granular pigment. Such misapprehension is, in point of fact, frequently made by the tyro in searching red blood corpuscles in moist blood films for malarial pigment.

It will be manifest from what has already been said in connexion with granular leucocytes, and crenated red corpuscles respectively (*Experiments* 3 and 4, *supra*), that the distinction between a dark element which is referable to pigment and a dark element which is referable to the deflection of light can generally be made by watching the effect produced by the alteration of the focus. Where the dark element corresponds to a point from which light is deflected, a change of the focus will be associated with a change from dark to bright. Where pigment is in question, a change of focus will substitute only a more diffuse for a less diffuse dark element.

(d) *Microscopic elements which resemble each other in contour and in refractive index cannot be discriminated from each in the microscopic outline picture.*

The difficulty of discriminating the different granulations in leucocytes in unstained blood films furnishes a familiar example of the above-mentioned imperfection in the outline picture.

(e) *The microscopic outline picture is imperfect in the respect that those elements which do not differ in refractive index from the medium in which they are imbedded are in every case unrepresented in the picture.*

The fact that bacteria may, when unstained, be invisible in the plasma or, as the case may be, in the interior of a phagocyte, and the fact that the nucleus may, when unstained, be invisible in the cell, will suffice to show the very formidable nature of the errors which the microscopist would fall into if he were to assume that the microscopical outline picture furnished to him a complete inventory of the microscopic objects contained in his preparation.

4. On the disadvantages and imperfections which attach to the microscopic colour picture.

The more conspicuous of the disadvantages which are associated with the microscopic colour picture are the following :—

(a) *In view of the fact that living objects must be killed and be subjected to the action of chemical reagents in the processes of fixing and staining, the picture which is obtained is in every case entirely artificial.*

(b) *Under the conditions of illumination particularized in Cap. I, subsect.* 7 (*B*), *there is a possibility of the observer lapsing into the fallacy of subjective colour contrast.*

The following may serve to illustrate the conditions under which this physiological phenomenon may mislead us into error in connexion with the microscopic image.

(*a*) The punctate areas of shadow which are developed by the deflection of the transmitted light from the irregular surface of the crenated red blood corpuscle may, in the case where the preparation is illuminated also by a bright top light, assume, in contrast to saturated yellow field which is provided by a mass of underlying red corpuscles, a bluish tint.

A failure to understand the genesis of this phenomenon may perhaps be responsible for the circumstance that a blue mottling on the red corpuscles was observed and placed on record by an eminent observer as a characteristic feature of malarial blood.

(*b*) Particles of dust and similar opacities scattered upon a stained microscopical specimen assume, when the preparation is illuminated by a bright top light, a coloration complementary to that of the background.

Where the colour which is thus generated is mistaken for objective colour, the particles in question may be mistaken for stained micro-organisms.

The error may in particular occur in connexion with a search for red-stained tubercle bacilli in the case where opaque particles are disposed upon a bluish or bluish-green microscopic field.

In this case and in all similar cases the differentiation between subjective and objective colour can be readily made if the fact is borne in mind that elements which are subjectively coloured will come out as black when the top light is screened off from the microscopical preparation.

(c) *Microscopic elements which resemble each other in contour and in their staining reactions cannot be discriminated in the microscopical colour picture.*

The fact that the agglomerations of basophil granules which occupy the protoplasm of the so-called " mast-cells " were for a long time confused with agglomerations of cocci, furnishes an illustration of this imperfection in the microscopic colour picture.

(d) *The microscopical colour picture is imperfect* (a) *in the respect that every object which fails to take the stain is unrepresented in the picture, and* (b) *in the further respect that only those objects which are differentially stained can be identified.*

The full import of these facts will only gradually be borne in upon the microscopical student.

In connexion with the study of bacteriology, he will, when he has completed his apprenticeship, begin to realize that the micro-organisms, of whose presence he is on other grounds assured, may quite well, where they have not been differentially stained, lie hidden in his preparation.

In like manner he will, in connexion with the study of pathological histology, in the case where he is dealing with preparations which are so stained as to reveal to him the changes in the tissues which are associated with bacterial invasion, recognize that the bacteria themselves will often be quite invisible. Again, he will find, in the case where his preparations are so stained as to depict the bacteria in a satisfactory manner, that the changes in the tissues will be displayed only very inadequately.

Lastly he will, in connexion with the study of histology, recognize that there are many elements in cell and intercellular substance which, in the ordinary preparation, withdraw themselves from observation, and which require for their display special staining methods.

5. General question of the limitations which are imposed upon microscopic achievement; and appraisement of the relative importance of each of the limitations which come into consideration.

While it is realized that certain limitations are imposed upon microscopic achievement, a great deal of confusion prevails with respect to the nature of these limitations.

The microscopist is in reality hampered—

(*a*) By the fact that he is in most cases limited to the employment of a particular type of stage picture.

The limitations which are imposed upon the microscopist in the matter of the selection of his object picture have been enumerated in *subsect.* 2, *supra.* They find illustration in the fact that we cannot in the case of diatoms resort to colour picture, and in the fact that we cannot in the case of microscopic sections bring into view bacteria except by resort to this type of picture.

(*b*) By the fact that the stage picture is never anything more than a very inadequate representation of the preparation under examination, and by the fact that there may be elements in the preparation which cannot be depicted at all in the colour picture.

Illustration has been furnished of this fact in *subsect.* 4 (*d*), *supra.*

(*c*) By the fact that certain optical defects manifest themselves in the microscopic image whenever very high magnifications are resorted to.

The difficulties which the microscopist has to deal with when dealing with very high magnifications will come under consideration in Part II of this Treatise.

The relative importance of the limitations which have been enumerated above cannot be finally appraised until we have completed that study of the microscopic image which lies before us in Part II of this Treatise. None the less, it will be well, before bringing to a conclusion Part I of this Treatise, which occupies itself with the object picture, to indicate the nature of the conclusion which will be borne in upon us.

That conclusion will be that the limitations which are imposed upon microscopic discovery by difficulties in connexion with the

achievement of an adequate representation of the object in the stage picture are out of all comparison more formidable than those which are associated with the adequate representation of that stage picture in the microscopic image.

We may already, to some extent, certify ourselves of the correctness of this conclusion if we consider the history of bacteriological research and seek to elicit in each case what is the factor which has determined the success or ill-success of the efforts which have been concentrated upon the disclosure of the causal agents of infective diseases.

Putting out of sight here everything which relates to the discovery of pathogenetic micro-organisms by the aid of methods of artificial cultivation, and confining ourselves to the question of the detection of micro-organisms in the infected organism, or in material derived from the organism, it may be asserted with confidence that where a micro-organism has been disclosed and identified in a case where it has been previously missed by generations of observers, that result has in no case been brought about by the achievement of a more adequate microscopic image of the stage picture. In every case it has been a more adequate representation of the object in the stage picture by a process of differential staining which has stood in direct causal relation with the disclosure and identification of the organism.

This sequence of cause and effect is illustrated in the case of the great advances in bacteriology which followed directly upon the introduction of the anilin dyes into histological technique. It is illustrated again in a conspicuous manner by the case of the discovery of the tubercle bacillus by Koch, which was confirmed by every bacteriologist by the aid of the improved method of differential staining which was immediately supplied by the ingenuity of Ehrlich. Finally, we have recent illustration of the same sequence of cause and effect in the discovery of the spirochaete pallida following upon the introduction of Giemsa's staining method.

If we pass from the history of the past to the consideration of the problems of the future, and in particular to the consideration of the fact that the causal agents of smallpox, scarlatina, rheumatic fever, measles, and many other human and animal diseases have not as yet been disclosed by the microscope, we find the following situation.

It has appeared to many who have considered the problem that

the cause of failure is here to be sought in an "ultra-microscopic minuteness" of the undiscovered micro-organisms. This is, as the reader will perceive, tantamount to assuming that the cause of failure is to be found in the inadequate representation in the microscopic image of elements which are adequately represented in the stage picture.

With regard to the correctness of this theory, it may be pointed out, in the first place, that the theory that the undiscovered germs of disease are of ultra-microscopic minuteness has at any rate no application in connexion with diseases such as smallpox, where the causal agents are confessedly large enough to be held back by the pores of the Chamberland filter. In the second place, the attention of the reader may be directed to the endeavour which is made in a subsequent chapter (Cap. XVI) to make it clear that the theory that objects may be too small to be imaged by the microscope is founded upon a confusion of terms and a misapprehension of the true bearing of certain optical facts.

To others, who have considered the fact that the microscope has failed to reveal the causal agents of many of our most familiar diseases, this failure appeals only as an illustration of the rule that micro-organisms (with rare exceptions) remain for all practical purposes invisible and unidentifiable in the interior of the organism until methods of differential staining are discovered which allow of their representation in the stage picture. If we have here, as the present writer believes, the true explanation of the present ill-success of the bacteriological microscopist in the matter of the discovery of the germs of the diseases specified above, that discovery cannot be expected until further progress shall have been made in those comparatively unregarded, but in reality fundamentally important, chemical researches which lead up to the invention of new processes of differential staining.

Part II.

THE MICROSCOPIC IMAGE AND ITS DEVELOPMENT.

CHAPTER VI.

IMAGE FORMATION BY THE SIMPLE APERTURE.

Function of the aperture in the formation of images—Conditions which determine the scale and brilliancy of the simple aperture image—Conditions which govern definition and resolution in plan in the case of the simple aperture image—Resolution in depth—Obstructive interference—Summary of the salient features of simple aperture images—Dilemma of the simple aperture image.

1. Function of the aperture in the formation of images.

Our study of image formation in the microscope may be conveniently prefaced by a consideration of the simpler cases of image formation.

It will help us to realize what is essential to an image if we set ourselves to consider wherein a blank luminous surface, such, for instance, as would be furnished by an illuminated white sheet of paper placed in front of us, differs from what we should characterize as *an image.*

The essentially important distinction is to be found in the fact that, while we have here, upon the white paper which serves as the receiving screen, light which is derived from all the luminous objects around, we have upon the screen, in the case where we are dealing with an image, light derived exclusively from a single object.

It is brought home to us by this consideration that the first —and indeed the only—essential to image formation is the isolation of the light of the object which is to be imaged from the light of the surrounding objects. The machinery by which the required isolation is effected is in each case a perforated screen. The perforation provides passage for the light which is to enter into the composition of the image, the substance of the screen excludes the extraneous light which would drown that image.

Having realized the function of the perforated screen in connexion with image formation, we pass to consider the conditions

which determine the magnitude, brilliancy and degree of resolution of the image.

The conditions which come here into consideration are :—

(*a*) The distance between the object and the aperture ;

(*b*) The distance between the aperture and the receiving screen ; and

(*c*) The dimensions of the aperture.

The influence of these different factors is disclosed in the experiments here subjoined.

2. Conditions which determine the scale and brilliancy of the simple aperture image.

Experiment 1. Take a large piece of cardboard, and having pierced it with a small aperture (a stout hat pin will make an aperture of suitable size) set up this screen at a distance of 60 cm. from a lighted candle. At the same distance behind the perforated screen set up a receiving screen. The image of the candle will then appear upon the screen somewhat as represented in Fig. 7.

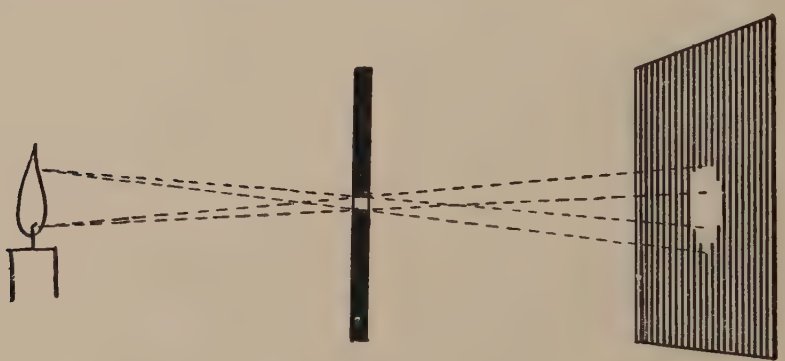


Fig. 7.

It will be noticed that the candle flame is here inverted. This inversion (which is a feature of every image formed through a single aperture) is the result of the crossing of the axes of the imaging beams in the centre of the opening.

It will further be observed that the image which is here formed corresponds in magnitude to the actual object.

The results of the above observations are in conformity with a law which we we shall find to hold true both in the case of the simple aperture and the lens-armed aperture. This law may be formulated as follows.

As is the distance from the object to aperture (object-aperture dis-

tance) to the distance from the aperture to the image (aperture-image distance), so is the magnitude of the object to the magnitude of the image.

Applied to the particular case under consideration, the formula will, with a vertical diameter of 5 cm. for the candle flame, give us a vertical diameter of 5 cm. for the candle-flame image.

	Object-aperture distance.		Aperture-image distance.			Size of object.		Size of image.
(*a*)	60 cm.	:	60 cm.	:	:	5 cm.	:	5 cm.

Experiment 2. Now move the perforated screen 20 cm. nearer to the candle, the receiving screen remaining in position.

BATTERSEA PUBLIC LIBRARY

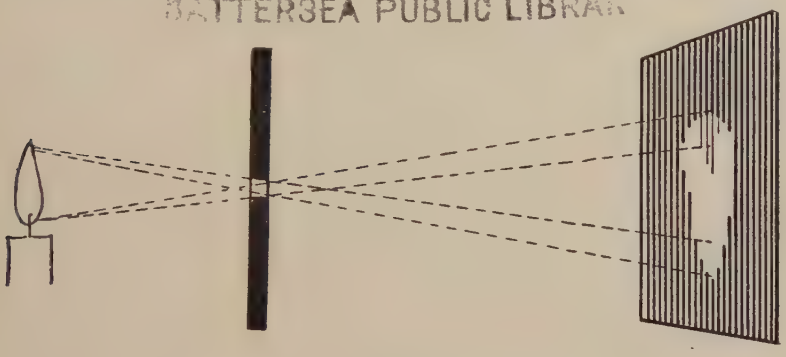


Fig. 8.

Note that the diameter of the object is now in conformity with the law given above, to the diameter of th e image in the proportion of 1 : 2

	Object-aperture distance.		Aperture-image distance.			Size of object.		Size of image.
(*b*)	40 cm.	:	80 cm.	:	:	5	:	10 cm.

The setting forward of the perforated screen will not only have increased the scale of the image, but it will have increased also the aperture of the imaging beams and in correspondence with this the total illumination. The average brilliancy of the image will, however, be unaltered, for the image is correspondingly magnified.

Experiment 3. Leaving now the perforated screen in position at a distance of 40 cm. from the candle, set back the receiving screen to a point 120 cm. from the aperture and 160 cm. from the candle.

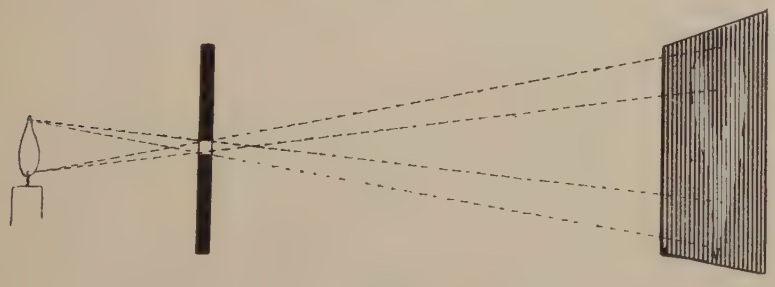


Fig. 9.

In conformity with the greater separation of the axes of the incident beams obtained as the aperture-image distance increases, the image will here be more highly magnified than in *Experiment* 2.

Our formula will tell us the exact increase of magnification :—

	Object-aperture distance.		Aperture-image distance.			Size of object.		Size of image.
(*c*)	40 cm.	:	120 cm.	:	:	5 cm.	:	15 cm.

As compared with the image in *Experiment* 2, the image here obtained will be less brilliant. This is in conformity with the greater total distance from candle to receiving screen and with the fact that illumination falls off proportionately to the square of the distance.

Experiment 4. Make now a second aperture in the receiving screen alongside of the first, and let it be considerably larger than the first, and now compare the image of the candle formed by the larger, with the image formed by the smaller, aperture.

It will be seen that in every position of the screen the larger aperture will give the same *net magnification* [1] as the smaller. There will, however, be a notable difference in the brilliancy of the images, the image formed by the larger aperture being in each case the brighter in the proportion in which the square of the diameter of the larger stands to the square of the diameter of the smaller, aperture.

3. Conditions which govern definition and resolution in plan in the case of the simple aperture image.

In the course of our study of the conditions which determine the scale and the brilliancy of the image, certain points in connexion with the *definition* [2] and *resolution* [3] of the image will have arrested attention.

It will have been noticed that in Fig. 7 the light of the candle is not entirely extricated from the light derived from surrounding objects. It is owing to this circumstance that the image shown on the screen still bears a general resemblance to the form of the aperture. It is not yet clearly *defined*.

[1] We may conveniently employ the term *net magnification* to denote the magnification measured on the receiving screen between the axes of the outermost incident beams. The *gross*, as distinguished from the *net magnification*, would be that measured by the extreme limits of the illuminated area on the screen.

[2] By *definition* is signified the sharp delineation of the image upon the screen, which results from the extrication of the light of the object which is to be imaged from the light derived from other sources.

[3] By *resolution* is signified that definition of separate elements in the image which results from resolving of the light which derives from an object as a whole into two or more components coming each from a different element in the object.

In Fig. 8 the image of the candle, seeing that it has lost all resemblance to the shape of the aperture, may be regarded as completely *defined*. Furthermore, it is already partially *resolved*, for it is now possible to distinguish in the image the apex from the base, and the darker centre from the brighter fringe of the flame.

In Fig. 9 *resolution* is carried a step farther. More detail has appeared in the image.

Fig. 10 below brings more plainly before the eye the phenomena of progressive resolution.

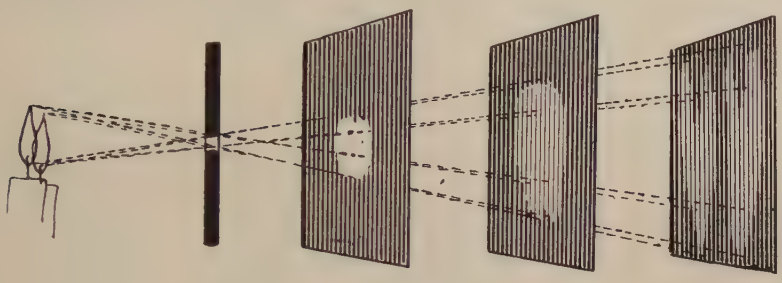


Fig. 10.

We have here a composite object constituted by two candles set up side by side in a plane parallel to that of the receiving screen.

In the first position of the screen there is represented an *undefined* image, which in its contour more closely resembles the aperture than the object which is to be depicted. In the second position of the screen, the image of the compound object is beginning to be *resolved* into the images of two separate candles. The images of these last are not yet clearly *defined*. In the third position of the screen the images of the individual candles are completely *defined* and are beginning to be *resolved*.

Fig. 11 (A and B) elucidates the manner in which these results are brought about.

In each case, as will be seen, three points in the candle flame are selected for consideration. In each case also the image of these points is represented as it would appear in three positions of the receiving screen.

To be noted is—first, that each point in the object is represented in the image by a diffusion disc ; secondly, that separate definition of the points is a question of the diffusion discs being spaced out sufficiently to avoid overlapping.

What particular amount of spacing out will suffice for this separate definition will obviously depend upon the relation in which

the diameter of the individual disc stands to the centre distance between the individual discs.

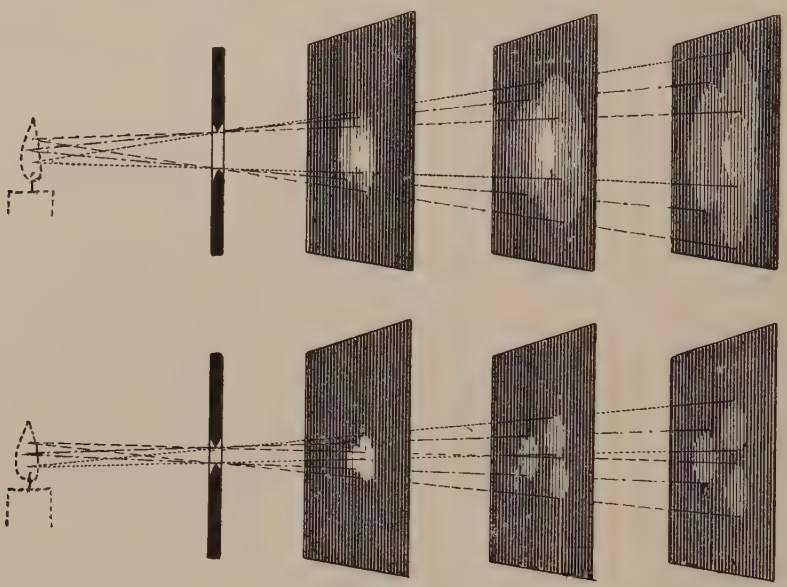


FIG. 11 (A AND B).

The conditions which govern the magnitudes of these measurements may be learned from a study of the diagram below.

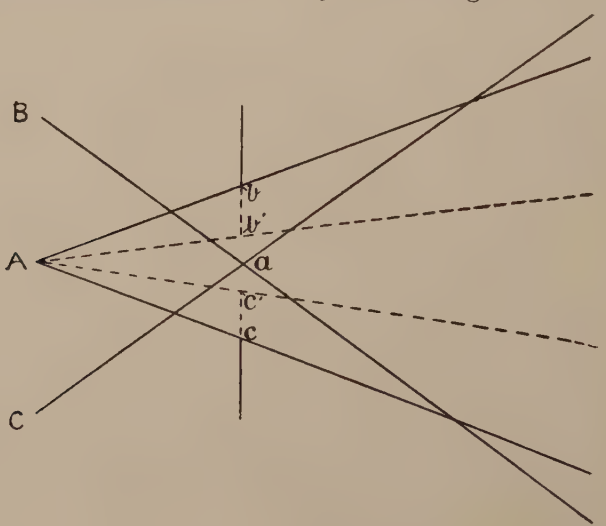


FIG. 12.

The *magnitude of the individual diffusion disc* is governed, (1) by the angle of aperture—*b A c*, *b' A c'*—according as we take into

account here the larger aperture *b c*, or the smaller aperture *b′ c′*, and (2) by the aperture-image distance. The former of these supplies the measure of the divergence of the individual beam at the point where it passes through the perforated screen. The aperture-image distance determines the further opening out of the beam behind that screen.

The *centre-to-centre distance between any two diffusion discs* is governed : (1) by the angle intercepted between the axes of the corresponding beams as these intersect in the aperture—*B a C*, in the figure,—and again (2) by the aperture-image distance.

The first measurement stands in relation with the object aperture distance and the linear distance between the points of origin of the beams which are under consideration ; the second measurement determines the extent to which these beams will separate behind the aperture.

Bringing now into relation with each other the law governing the size of the diffusion disc with the law governing the spacing out of points upon which the separate diffusion discs are centred, we arrive at the law which governs the resolution of the simple aperture image.

This law may be expressed as follows :—

For the separate definition in the image of any two points in the object, it is essential : first, that the "separation angle"—i.e. the angular distance between lines drawn from the points in question to the centre of the aperture—shall be greater than the angle of aperture, and, secondly, that the receiving screen shall be set back sufficiently to allow of the corresponding beams extricating themselves completely from each other.

The series of diagrams—Figs. 7–11—which we have already considered in connexion with the magnification of the image, furnish a series of simple illustrations of the law of resolution as formulated above.

Commencing with Fig. 11 B, we see that we have here, in the circumstance that the angular distance between the points is greater than the angle of aperture, an operative factor which determines that the separation of the axes of the incident beams shall proceed more rapidly than the opening out of the component rays of the individual beams.

We have in association with this the circumstance that in the second and third positions of the receiving screen, the beams from the separate points reciprocally extricate themselves from each other.

The resolution which is obtained is thus in conformity with the law just formulated.

The progressively improving resolution in the three positions of the screen in Fig. 10, and the improved resolution obtained in Fig. 9, as compared with Fig. 8, by the setting back of the receiving screen, is the result of the operation of precisely similar causes.

A study of Figs. 7 and 8 shows that in each case the angle of aperture is here smaller than the angle of separation between the upper and lower points of the flame. Thus, in each case we have the conditions under which the setting back of the receiving screen would give better resolution.

Fig. 11 A illustrates the fact that resolution may be impaired by the setting back of the receiving screen.

The fact that the diameter of the aperture is here greater than the linear distance between the points in the object gives us an angle of an aperture which is greater than the separation angle.

This imports that the linear distance between the delimiting rays of the individual beams will, behind the aperture, increase more rapidly than the linear distance between the axes of those beams. In association with this will be the progressively greater overlap and the progressive impairment of resolution shown on the second and third positions of the receiving screen.

Summarizing the teaching of the diagrams, we arrive at the following—

(1) The setting back of the receiving screen from the aperture will improve or impair resolution according as the separation angle is greater or less than the angle of aperture.

(2) The limit of resolution for a particular aperture will have been reached when elements which are in the object separated by a distance just greater than the diameter of the aperture have been separately defined.

(3) Where this limit has been reached an improvement in the direction of further resolution can be obtained only by diminishing the size of the aperture.

4. Resolution in depth.

The problem of resolution was considered in the last section only in its relation to the separate definition in the image of elements disposed one beside the other on the same optical plane in the object.

We have to consider, in addition, the question of *resolution in*

depth, i.e. the question of the separate definition in the image of elements, which in the object lie directly one behind the other upon different optical planes.

Such resolution is not obtained in the simple-aperture image.

To see to what extent the quality of the image may be impaired by defect of resolution in depth, we may take the case of a compound object whose constituent elements are disposed on widely separated planes.

We may take, for instance, the case where we have a single candle set up in close proximity to the aperture, and some distance behind, two other candles. In this case the image of the object is confused by the superposition of the images of the further candles upon the image of the nearer candle (Fig. 13).

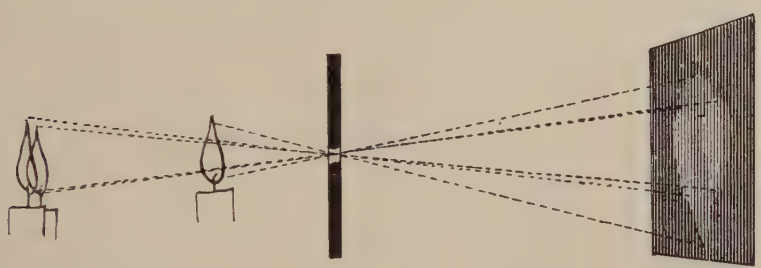


Fig. 13.

When we look into the cause of this confusion we recognize that this confusion of nearer and further is in reality only a special case coming under the general law, that separate definition of two objects is obtained only when the beams from those points separate out from each other.

5. Obstructive interference.

Closely associated with the question of resolution in depth is that of the effect exerted upon the image by obstructive interference. It will suffice for the present to note that the interposition of an opaque element in any position between the radiant object and the receiving screen will involve the projection of a dark shadow upon the picture and the blotting out of so much of the object as is subtended by the obstacle.

6. Summary of the salient features of the simple-aperture image.

The achievement of a satisfactory image of any minute object involves compliance with the conditions which contribute respec-

tively to high magnification, brilliancy, resolution in plan, and resolution in depth.

Magnification. We have seen that the size of the object is to the size of the image as the object-aperture distance is to the aperture-image distance.

In conformity with this, any desired magnification can be obtained in the simple aperture image by an adjustment of the distances between the object, the aperture and the receiving screen.

Brilliancy. The brilliancy of the image—meaning thereby, its average brilliancy per unit of area—is governed by (*a*) the luminosity of the object ; (*b*) the dimensions of the aperture, and (*c*) the object-image distance—i.e. the total distance, measured from the object to the receiving screen.

The luminosity of the object remaining the same, the brilliancy of the image will vary, directly as the size of the aperture, and in accordance with the law of inverse squares with the object-image distance.

The achievement of better illumination thus involves a setting forward of the receiving screen and an increase in the dimensions of the aperture.

Resolution. Representation in the case of the simple aperture image is, as we have seen, always a question of the representation of points by diffusion discs.

Separate definition of the points or larger features of the object is obtained only in the case when the angle which these subtend at the aperture is greater than the angle of aperture, and when the receiving screen is set back to the point at which the beams which take origin from the elements in question have extricated themselves from each other.

In accordance with this it is impossible in the simple-aperture image to obtain separate definition of points which are separated by a linear distance less than the diameter of the aperture.

A fortiori it is impossible to obtain separate definition of points lying one directly behind the other.

It is also impossible to bring into view any object which is disposed behind an opaque obstacle.

7. Dilemma of the simple-aperture image.

Taking a general survey of the conditions set forth above as essential to the achievement of adequate magnification, brilliancy, and resolution in the simple aperture image, it will be appreciated that these are mutually exclusive.

Attention may be concentrated upon the circumstance that illumination such as will permit of the employment of high magnifications can be obtained only with a large aperture, while satisfactory resolution can be obtained only by the employment of a small aperture.

We are here obviously face to face with the alternative that we must either sacrifice resolution to illumination, or illumination to resolution. We may conveniently think of this as *the dilemma of the simple-aperture image.*

We shall see in the next chapter how, by the use of a lens, a way of escape is found both from this dilemma, and from that confusion of nearer and further, which is, as we have seen, inseparable from the simple-aperture image.

CHAPTER VII.

ON IMAGE FORMATION BY THE LENS-ARMED APERTURE.

Introductory—Resemblances between the image furnished by the "simple" or "vacant aperture," and the image furnished by the "lens-armed," aperture—Differences between the simple aperture image and the lens image—Separate definition of elements placed side by side upon the same object plane—Separate definition of elements placed one behind the other on different focal planes—Imaging of elements in the object which are concealed from direct view by intervening obstacles.

Conception of a "VISTA."

"POINT VISTAS"—*Dimensions which come into consideration in the case of a "point vista" and method of measuring and expressing these dimensions—"Angular aperture"—"Numerical aperture"—Examples of the method of calculating angular aperture and numerical aperture—Connexion between the configuration of a vista and the quality of the image obtained—Relation between aperture and the greater or less brilliancy of the point image—Relation between the aperture and the greater or less sensitiveness of the image to alterations of the focussing adjustment—Relation between the aperture and the greater or less liability of the image to occultation by intrusive elements—Conception of interpenetrating point vistas—Influence of the aperture upon the definition of the focal images in the case of intrusive vistas.*

"SURFACE VISTAS"—*Configuration of* (a) *the simple magnifying vista, of* (b) *the simple minifying vista, and* (c) *of the vista in which magnification is followed by minification—Dimensions which come into consideration in the case of surface vistas—Relation between the configuration of the surface vista and the scale of the image—Examples of the application of the formulæ for the determination of the magnification of the image—Extent of the object field which is imaged—Examples of the application of the formula for determining the extent of the object field—Quality of the image—Influence exerted by the aperture of the vista upon resolution in plan—Influence exerted by the aperture of the vista upon resolution in depth—On the aperture of the vista as a factor which determines the extent of the plane of origin occulted by an intrusive element and the more or less satisfactory representation in the image of partially eclipsed elements.*

Conception of a VISTA *as a sequence of radiant planes.*

Conception of a CATENA OF VISTAS—*Image formation in a catena of surface vistas—Law governing the inversion of the image in a catena of surface vistas—Methods of measuring the scale of the image obtained on the terminal plane of a catena of vistas—Determination of the scale of the terminal image of a catena of vistas by dividing the numerical aperture of the opening angle* (*N.A.*) *by the numerical aperture of the closing angle* (*n.a.*) *—On the imaging of the successive optical planes of the original vista—Imaging of the apertural plane of an antecedent vista in the apertural planes of all the following vistas—Preliminary indications of the practical applications of the fact that the field upon which is imaged the apertural plane of the antecedent vista corresponds in area and position with the apertural plane of the following vista—Exploitation of the circumstance that the sectional measurement of the image of the aperture of the antecedent vista furnishes the sectional measurement of the Ramsden disc of the following vista—Exploitation of the circumstance that in a catena the apertural plane of every antecedent vista is imaged and available for scrutiny in the Ramsden disc of the terminal vista—Association of progressive restriction of the aperture with cumulative magnification, and preliminary indications of the significance of this progressive restriction of the aperture in connexion with the development of a critical image, and the limit of microscopic resolution.*

1. Introductory.

We may most conveniently pass from the study of image formation by the vacant aperture, to the study of image formation by means of the lens-armed aperture, by comparing the course and configuration of a beam of light transmitted in a symmetrical manner through a lens-armed aperture with the course and configuration of a beam transmitted through the same vacant aperture.

The *course* of the beam, meaning thereby in each case the course followed by the axial ray which traverses the centre of the aperture, will in the two cases be essentially the same.

The *configuration* of the beam will, on the contrary, be very different. In the case of the vacant aperture the beam will, as is shown in Fig. 14, A, be represented upon the screen by a luminous disc which will expand as the distance between the receiving in screen and the aperture increases.

In the case of the lens-armed aperture, the beam may be laid down upon the screen according to circumstances, in the different forms indicated in Fig. 14, B, C, and D, and again with more detail in Figs. 15, 16 and 17.

In Fig. 14, B, and Fig. 15, we have a beam originating at a point lying between the principal focus upon the surface of the lens. The diagram shows that the delimiting rays—and what holds true of these holds true of all the included rays—are bent in by the lens in such a manner as to diminish the angle of divergence of the beam.

In conformity with this, the beam would be laid down upon the screen at progressive distances from the aperture in the form of diffusion discs, which are, as a comparison with those in Fig. 14, *a*, reveals, in each case smaller than those which would be obtained with an unarmed aperture of equal diameter.

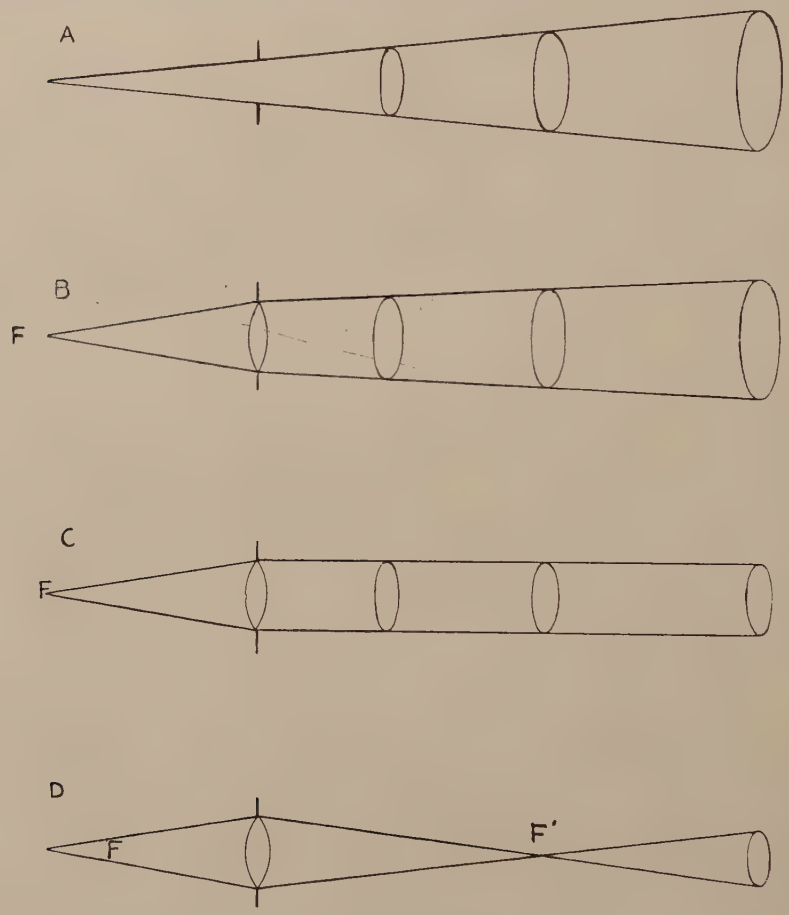


Fig. 14.

In Fig. 14, *c*, and Fig 16, we have in each case a beam taking origin in the principal focus of the lens. In each case the rays which diverge from the radiant point are rendered parallel by the lens, with the result that the point is represented upon the screen set up at progressive distances from the aperture by a succession of discs of uniform size.

In Fig. 14, D, we have a beam taking origin beyond the principal focus of the lens. Here the divergent rays are rendered convergent

in such a manner as to intersect with the axial ray and to diverge again behind the conjugate focal point (F, in Fig. 14).

In conformity with this, in Fig. 17 the beams are laid down upon the nearest screen in the form of discs, upon the middle screen in the form of points, and on the farthest screen in the form of discs.

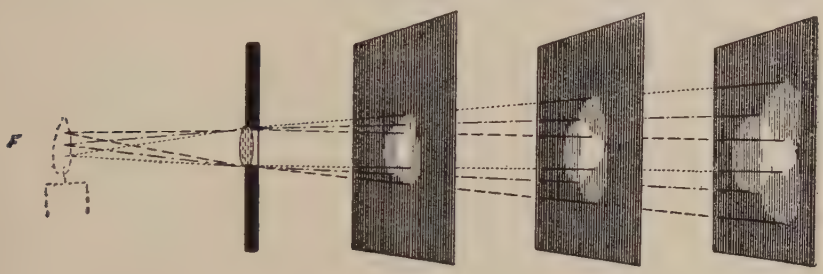


FIG. 15.

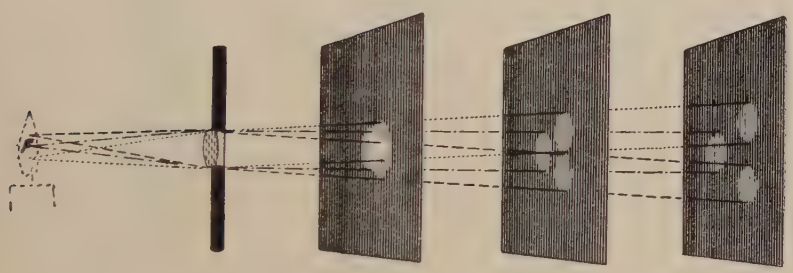


FIG. 16.

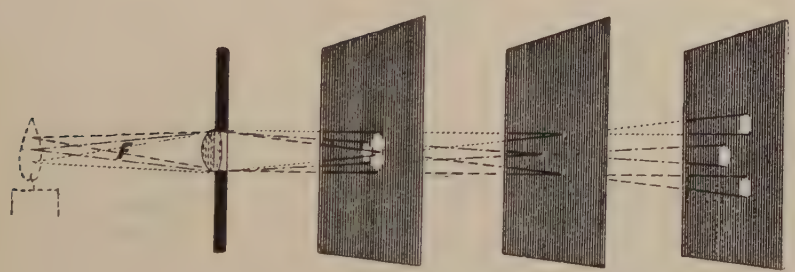


FIG. 17.

2. Resemblances between the image furnished by the "vacant" and the image furnished by the "lens-armed" aperture.

The image furnished by the lens-armed aperture resembles that furnished by the vacant aperture:

(*a*) in the respect that it is in each case an inverted image, and

(*b*) in the respect that it is—given the case where the object,

aperture, and receiving screen occupy in the two cases the same relative positions—an image on the same scale.

The corollary that the lens is, from the point of view of the achievement of magnification, absolutely inert, is so fundamental and, at the same time, so unfamiliar, a proposition, that it will be profitable for the reader to convince himself of its truth by undertaking the following experiment.

Experiment. Take a piece of cardboard perforated by an aperture just large enough to admit the point of a pencil. Set up this perforated screen in front of two lighted candles.

Now let these two conjointly do duty as an object, and let the distance between the two represent the dimensions of the object.

Covering the aperture of the perforated screen with the convex lens, bring a receiving screen into position so as to obtain at the con-

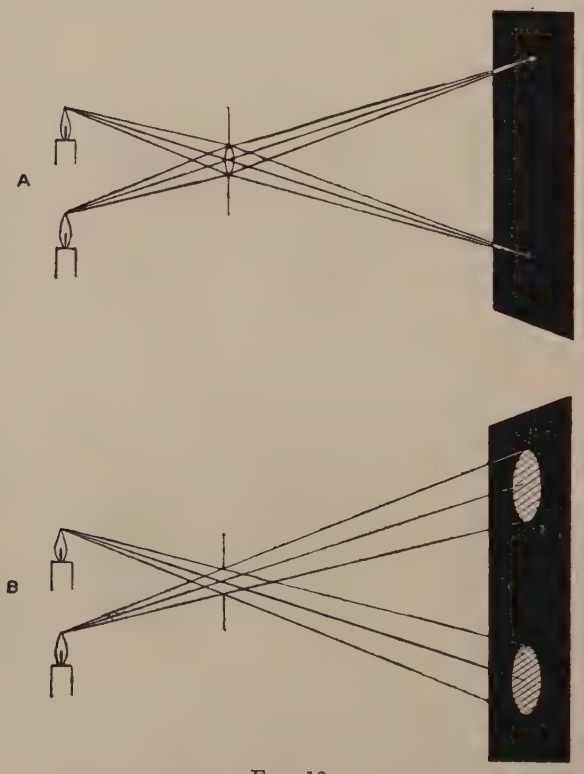


Fig. 18.

jugate focal distance a sharply focussed image of the candles. Taking off from the centres of the individual images, measure by the aid of a pair of calipers the distance between the mid points of the candles in the focussed picture.

Now take away the lens and, keeping everything else in place, go

over the measurement again upon the simple-aperture picture, taking off, as before, from the mid points of the imaged candle flames.

The measurement which represents the dimensions of the image furnished by the vacant aperture will be found to be practically the same as the measurement which represents the dimensions of the lens image.

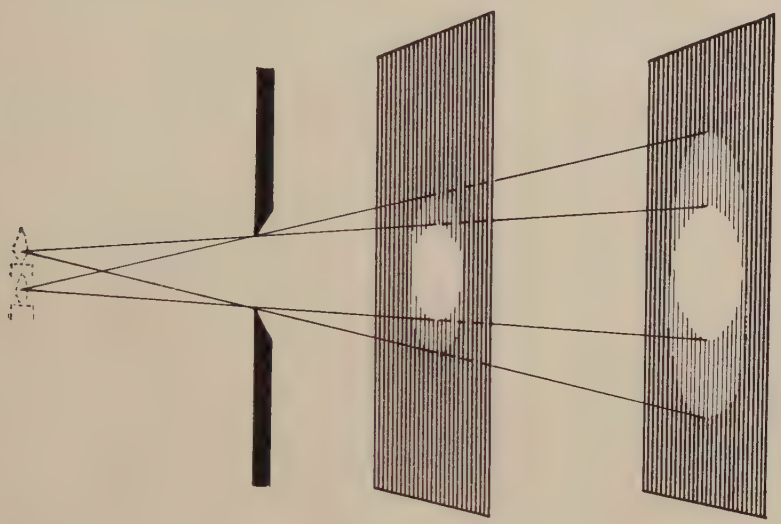


FIG. 19.

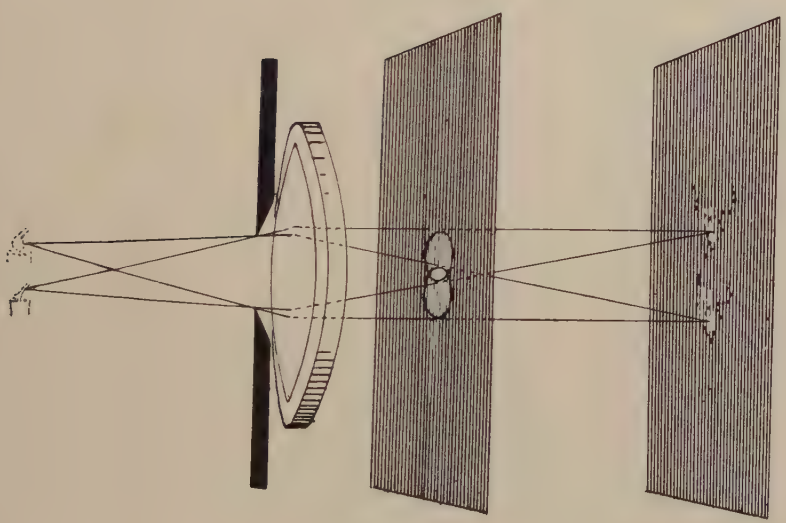


FIG. 20.

Fig. 18, which takes into consideration only the beams from the tips of the candle flames, will serve to elucidate the experiment.

3. Differences between the simple-aperture image and the lens image.

Differences emerge between the lens image and the simple-aperture image in the matter of—

(*a*) The resolution of the image in plan ;

(*b*) The resolution of the image in depth ; and

(*c*) The imaging of the elements of the object which are screened from direct view by interposed objects.

4. Separate definition of elements placed side by side upon the same object plane.

Fig. 14 D, and the experiment in *subsection* 2, *supra*, have brought it home to us that the lens when disposed at an appropriate distance from the object is capable of dealing with beams taking origin from that object in such a way as to concentrate the system of rays which proceeds from a radiant point upon a focal point in the receiving screen. The superiority which this gives to the lens image over the simple-aperture image, from the point of view of the resolution of elements placed side by side in the object, comes very strikingly before the eye on comparing the two diagrams (Figs. 19 and 20). In contrast with what occurs in the case of the simple-aperture image, we see that the beams in the case of the lens image extricate themselves completely from each other, giving us in the conjugate focal plane separate definition of each point in the object.

5. Separate definition of elements placed one behind the other on different focal planes.

By the fact that the lens effects in the case where the receiving screen is placed in the conjugate focal plane a concentration of every ray of the beam upon its axial ray, there is secured in the case of the lens image not on'y definition in plan but also definition in depth.

The difference which obtains in this respect, as between the lens image and the simple-aperture image, emerges on comparing Fig. 21 with Fig. 22, which differs from it only in the respect that the lens has been imposed upon the aperture. While in the case of the simple-aperture image the points A and B, placed directly one behind the other on the line of collimation, and C, disposed vertically above that line at a distance less than the diameter of the aperture, are represented in the image by a system of overlapping diffusion discs, the points A, B, and C are in the case of the lens image separately

defined. The definition is, in the case of each of the points, definition upon the field which is furnished by the diffusion disc or discs, which represent the out-of-focus element or elements.

This kind of definition is, in the case where the dimensions of the diffusion discs are considerable, for all practical purposes equivalent to definition upon a uniform feebly illuminated field.

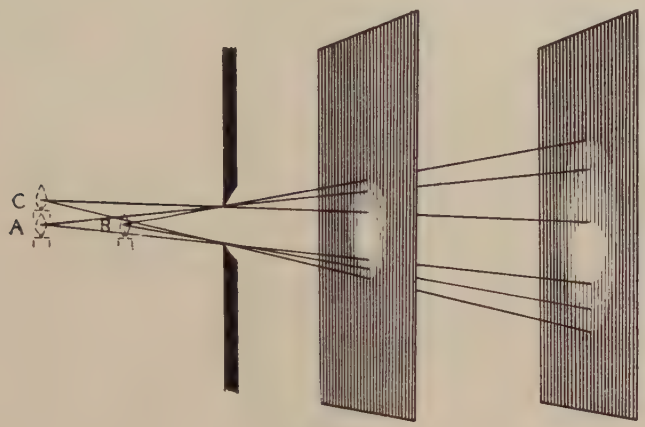


Fig. 21.

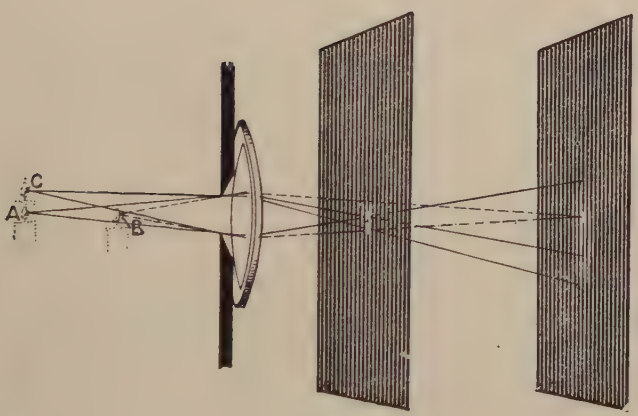


Fig. 22.

We see on the first receiving screen (Fig. 22) that the beam from B is still represented by a diffusion disc when the beams from A and C are concentrated upon a point. We see on the second receiving screen that when the beams from A and C have opened out again behind their focal points the beam from B is, in its turn, concentrated upon a point.

6. Imaging of elements in the object which are concealed from direct view by intervening obstacles.

In addition to points of difference considered in *subsections* 4 and 5 there is a further fundamentally important point in which the pictures produced by the agency of a lens differ from the pictures produced by a vacant aperture. A different effect is produced in the two cases by the interposition of an obstacle in the direct line between the radiant point and the receiving screen.

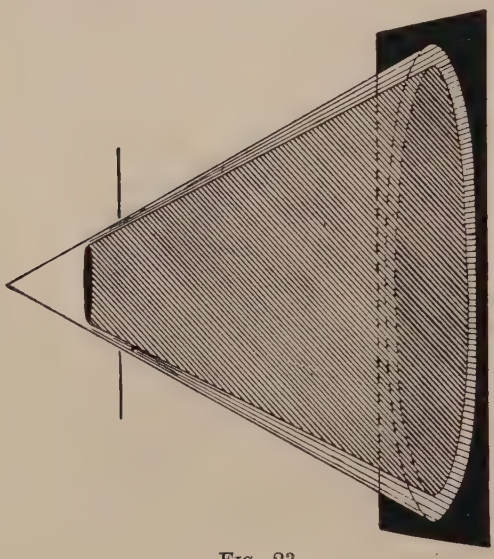


Fig. 23.

The figures below will elucidate this difference. It will be seen that, in the case where the simple aperture is employed, the partial obstruction of the beam involves the projection upon the screen of an expanding cone of shadow and a notable disfigurement of the image.

Where a lens is employed the partial obstruction of the beam involves nothing more than a reduction of the luminosity of the image. The radiant point is, as is shown in the diagram, still imaged in proper form and in proper position on the receiving screen by the agency of the circumventing rays, which come into focus from the peripheral zone of the lens.

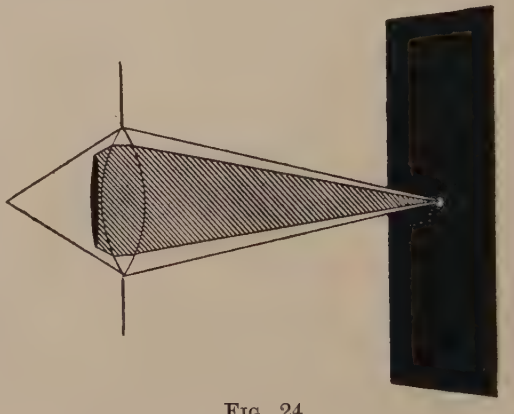


Fig. 24.

It will be convenient at this stage, before going more deeply into the questions which arise in connexion with lens images, to possess ourselves of a conception which will facilitate the discussion of the optical problems before us.

7. Conception of a "vista."

The exposition of the manner in which an image is built up in an optical instrument such as the microscope is encumbered by the circumstance that the optical element which has been considered has always been the individual ray.

So large a number of separate rays have to be considered, and so complicated and so diverse is the course of each of these rays, that it becomes a matter of difficulty to achieve any adequate mental picture of the aggregate of luminous impulses which are passing up through the instrument.

A way of escape out of this difficulty may be perhaps found by dealing with a larger optical unit than the ray. We may conveniently — as was suggested to me by my friend Mr. Gordon—take as our optical unit the figure which is traced out in an optical instrument by the path of a luminous impulse from a focal point of origin to its conjugate focal point.

We may speak of this optical element as a *vista*, or, more specifically, as a *point vista*.

It will be profitable to familiarize ourselves with the configuration and structure of the different varieties of "point vistas."

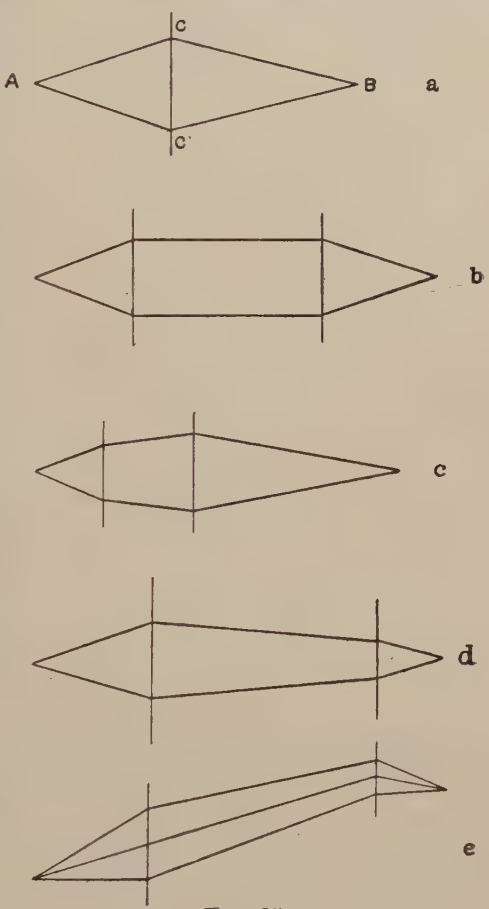


FIG. 25.

8. Point Vistas.

(1) The lozenge-shaped figure (Fig. 25, *a*), which is delineated when a beam enters a lens and is focussed by it upon a point, constitutes what we may speak of as the type form of point vista.

We may distinguish in it :

(*a*) The pole of origin, A.

This corresponds to the radiant point from which the beam of light proceeds.

(*b*) The opening limb, with its included opening angle, C, A, C′.

This corresponds to the diverging pencil of light which passes into the lens.

(*c*) The equator or waist, C, C′, where all the component rays run a course parallel to the axis of the beam.

This region corresponds, in the case of the simple convex lens here under consideration, with its principal plane.

(*d*) The closing limb with its included closing angle, C, B, C′.

This corresponds to the pencil of light which converges upon the focal point.

(*e*) The terminal pole, B.

This corresponds to the focal point.

The above "type form" of the point vista is diverged from under certain conditions.

(2) Where the diverging beam is rendered parallel by the first lens, and is brought to focus by the agency of a second lens, we have to deal with a vista such as that represented in Fig. 25, *b*.

The conical extremities of the figure here constitute the opening and closing limbs of the vista. The waist or region of parallel rays is represented by the cylindrical region of the beam which intervenes between these conical extremities.

(3) Where a beam emerges from the front lens of a combination in the form of a diverging beam and is afterwards focussed by a second lens, its configuration is as represented in Fig. 25, *c*.

In such a vista the waist is represented by a mathematical plane positioned in the back lens.

(4) Where a beam which has already been rendered convergent is brought to a nearer focus by the aid of a supplementary lens, its closing limb assumes, in the case where it lies in the axis of collimation, the blunted contour shown in Fig. 25, *d*. In the case where the beam falls eccentrically upon the supplementary lens it assumes the incurved contour shown in Fig. 25, *e*.

9. Dimensions which come into consideration in the case of a point vista, and method of measuring and expressing these dimensions.

In connexion with every point vista we have to consider the length of the opening and closing limbs, the diameter of the waist, and the ratio which the diameter of the waist bears to the length of the opening and closing limbs respectively.

Dimensions of the opening and closing limbs. In the case of a "point vista" the distances A–C, C–B, in Fig. 26 below, along the confines of the vista, not A–C′, C′–B, the distances measured along the axis, are taken as measuring the object-aperture and the aperture-image distances. The adoption of the former instead of the latter measurements is dictated by considerations of convenience, it being more convenient to substitute for the measurement of an axial path which lies partly in air and partly in the denser medium of the lens, a measurement of an outlying path which may be assumed to lie entirely in air.

Such a path, though appearing in the case of a diagram as a path longer than the axial path, is, in accordance with the principle that all paths from focal point to focal point are equal each to each, in reality optically of the same length.

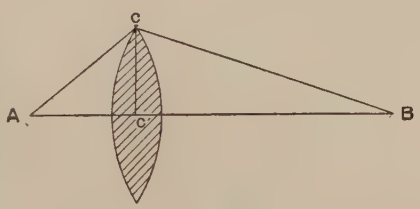


FIG. 26.

Dimensions of the aperture. When we speak of the aperture of the vista, we have for the most part in view not the linear aperture (i.e. the diameter of the waist), but the angular aperture.

10. Angular aperture.

Angular aperture may, for the purposes we have here in view, be defined as the ratio which the semi-diameter of the waist bears to the opening or, as the case may be, to the closing of the limb of the vista. It is obtained by dividing the semi-diameter of the waist (C–C′) by the measurement of the opening or, as the case may be, of the closing limb of the vista (A.C. or B.C. as the case may be).

The expression thus obtained is technically styled the sine of the *divergence angle*—the divergence angle being half the angle enclosed within the opening or, as the case may be, the closing limb of the vista.

11. Numerical aperture.

When it is desired to institute comparisons between the dimen-

sions of an angle measured in air and the dimensions of another angle measured in another medium—for instance, in oil or water—we employ for the purpose of the comparison *numerical aperture*, as distinguished from simple *angular aperture*.

Put otherwise, we refer our measurements to a common standard by multiplying the angular aperture (obtained as explained in *subsect.* 10) in each case by the refractive index of the medium in which the focal length has been measured.

The expression thus obtained :—*sine of divergence angle multiplied by the refractive index of the medium in which the focal length has been measured*—is what is understood by the expression *numerical aperture*.

It is conventionally denoted by the symbol N.A. We shall find it convenient, with a view to distinguish in each case between the numerical aperture of the opening limb and the numerical aperture of the closing limb, to employ, in the first case, the symbol—N.A., in the second case, the symbol—n.a.

The real significance of numerical aperture will appear on consideration of the following :—

We saw that the expression $\frac{\textit{semi-diameter of the waist}}{\textit{object-aperture distance}}$ represents the angular aperture of the opening limb of the vista.

What we proceed to do when we substitute for this angular aperture the numerical aperture is to increase, in the case where an immersion fluid of higher refractive index is substituted for air, the value of our fraction. This result is achieved by multiplying the numerator by the factor corresponding to the shortening of the focal distance which would have been effected if the measurement of this distance had been made in air instead of in the immersion fluid.

The justification for the statement that the focal distance would have been shorter if it had been measured in air will appear on following out, in connexion with the diagram below, the following simple train of reasoning.

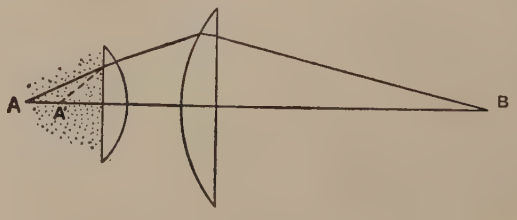


Fig. 27.

Conceiving here of a vista in the diagram as having its pole of origin in the conjugate focal point B, on the right hand of the figure, and of the luminous impulse as carried backwards from this point to its conjugate focal point in front of the lens, and following the course of the delimiting ray, we see that it is refracted on entering and leaving the back lens, that it again undergoes refraction on entering the front lens, and that it finally issues on the front surface of the lens into the homogeneous immersion fluid without further refraction.

It will be manifest that the conjugate focal point A, which is arrived at in such a case is more remote from the lens than the conjugate focal point A′, which would have been arrived at if additional refraction had taken place at the front face of the lens, as would have happened if the beam had emerged from glass into air.

The increase of the value of the fraction by a factor corresponding to the difference between the shorter and the longer focal distance finds in this consideration its justification.[1]

12. Examples of the method of calculating simple angular aperture and numerical aperture.

Example 1. The linear semi-aperture of the vista is 5 mm., the length of the opening limb of the vista measured in air is 16 mm.

The angular aperture of the opening limb is accordingly $\frac{5}{16}$ (0·31).

The numerical aperture of the opening limb (N.A.) is in this case identical with its simple angular aperture, inasmuch as the measurement of the opening limb is undertaken in air, i.e. in a medium whose refractive index is represented by unity.

Example 2. The linear semi-aperture of a vista is 5 mm. The length of the closing limb measured in air is 80 mm.

The angular aperture of the closing limb is accordingly $\frac{5}{80}$ (0·06).

The numerical aperture of the closing limb (n.a.) is again identical with its simple angular aperture.

Example 3. The linear aperture of a vista is 4 mm. The length of the opening limb measured in oil is 3 mm.

The simple angular aperture is $\frac{2}{3}$ (0·66).

To obtain the N.A., we diminish the length of the opening limb in the proportion in which it would have been diminished if it had been measured in air.

We effect our purpose in the most convenient manner by multiplying the numerator by 1·5—the refractive index of the oil:

$$\text{N.A.} = \frac{2 \times 1{\cdot}5}{3} = 1.$$

[1] For proof of the proposition that the focal lengths of the lens, as measured respectively in air and in an immersion fluid, are related to each other as the refractive indices of these media, the reader is referred to special optical treatises.

13. Connexion between the configuration of a vista and the quality of the image obtained.

Consideration teaches us that the conceptions of magnification and inversion which suggest themselves in connexion with images taken generally, have no application in connexion with the image formed at the terminal pole of a point vista.

The image of a point cannot be conceived of as either erect or inverted, or as diminished or magnified.

There are, however, other properties which may be predicated of a point image.

(*a*) The image may be more or less brilliant.

(*b*) It may be more or less sensitive to an alteration of the focussing adjustment.

(*c*) It may be more or less liable to occultation by elements intruding into the vista.

(*d*) It may be more or less distorted by spherical and chromatic aberration.

(*e*) Lastly, it may be more or less complicated by diffraction phenomena.

Reserving until later (*Caps.* VIII and IX) the consideration of the disturbances introduced into the point image by chromatic and spherical aberration and by diffraction respectively, we may consider here only the relation between the aperture of a vista and (*a*) the brilliancy of the image ; (*b*) its sensitiveness to inaccuracy in the focussing adjustment ; and (*c*) its liability to occultation.

14. Relation between aperture and the greater or less brilliancy of the point image.

Consideration will show that the luminosity of the image will be influenced by the aperture of the vista. Where much light is gathered in by the lens the image will obviously be more brilliant than in the case where the aperture is narrow and little light is gathered in.

15. Relation between the aperture and the greater or less sensitiveness of the image to alterations of the focussing adjustment.

Fig. 28 below will make it manifest that in the case where we are dealing, as in B, with a vista possessing only a small angular aperture a relatively large excursion of the focus will exert comparatively little effect on the superficial area of the image (diffusion disc) laid down upon the receiving screen. When, on the contrary, as in A, we are dealing with a vista possessing a wide angular aperture,

a very small excursion of the focus will transform the focal-point image into a diffusion disc of very sensible dimensions.

A moment's consideration will make clear the import of these facts. It will make clear that the greater the change effected in the image by a minimum excursion of the focus the more accurately will it be possible to locate the optical plane upon which the focal point is disposed. Further, it will bring it home to the mind that sensitiveness to alteration of the focussing justment is the correlative of the achievement of resolution in depth.

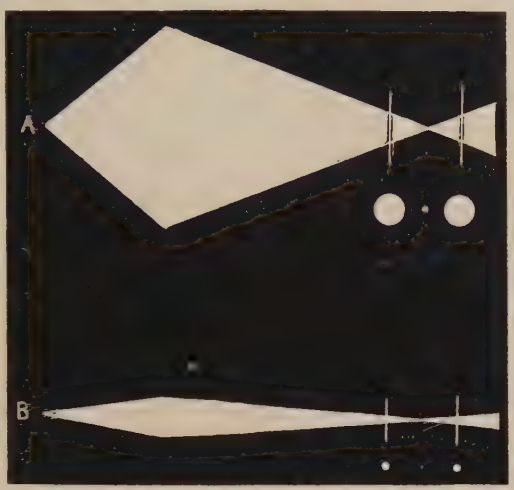


Fig. 28.

16. Relation between the aperture and the greater or less liability of the image to occultation by intrusive elements.

The effect exerted on the image by an intrusive element will, according as that element is positioned in the opening limb or closing limb of the vista, be determined by the sectional measurement of that opening limb, waist, or closing limb. Fig. 29 makes it plain that an obstacle which, placed in the waist of a narrow-angled vista—*a*—occults the image or, as the case may be, darkens it down appreciably, effects, when placed in the waist of a wide-angled vista—*c*—only an insignificant reduction of the brightness.

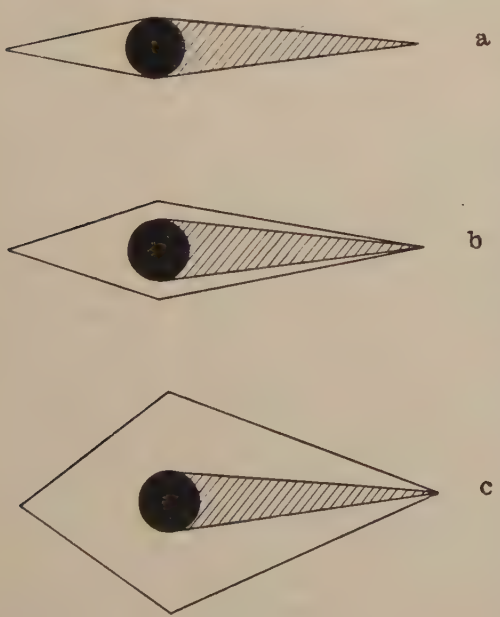


Fig. 29.

What applies to the case of intrusive obstacles in the waist of narrow and wide-angled vistas respectively, will manifestly apply with even greater force to the case of intrusive obstacles positioned in the neighbourhood of the poles of such vistas.

17. Conception of interpenetrating point vistas.

An isolated point vista, such as has up to the present been considered, could present itself only in the case of a radiant point set up in front of a lens in complete darkness and vacancy. In every other case we have, in addition to the point vista primarily under consideration, other point vistas taking origin from radiant points, disposed upon the same optical plane, or disposed, as the case may be, one behind the other upon different optical planes.

Where a system of beams taking origin from points disposed upon the same optical plane comes under consideration, we may speak of this aggregate of point vistas as a *surface vista.*

Where beams taking origin from points disposed one behind the other on different optical planes are focussed by a lens upon a succession of conjugate focal planes we may speak of these beams as constituting *interpenetrating vistas.* We may also speak of the vistas which interpenetrate with the vista primarily under consideration as *intrusive vistas* (Plate VI, A and B).

18. Influence of the aperture upon the definition of the focal images in the case of intrusive vistas.

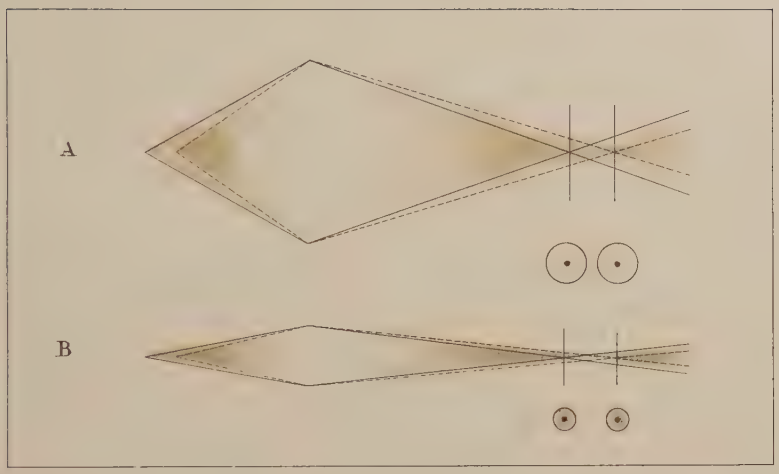
We have seen that resolution in depth is always a matter of the definition of point images upon the field furnished by the optical sections of the interpenetrating vistas.

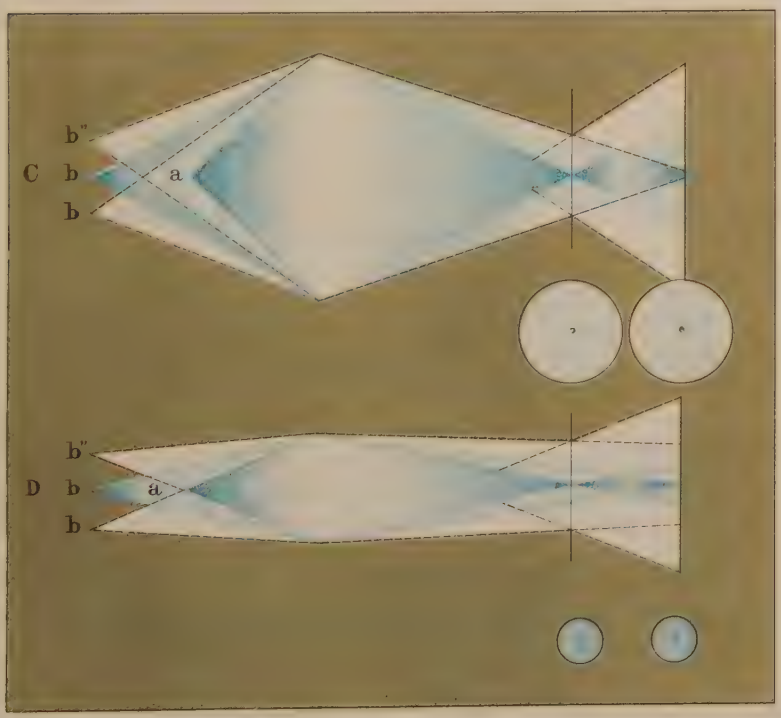
The definition will manifestly be satisfactory or unsatisfactory according as the luminosity of the point image differs more or less noticeably from the luminosity of the diffusion disc which provides the enveloping field. The luminosity in question will, as consideration will show, in every case be inversely as the superficies covered by the unfocussed beam.

It follows that clear definition of points on the same line of allineation will depend directly upon the aperture as determining the configuration of the vistas which come to focus before and behind the vista which is under consideration.

The diagrams A and B on Plate VI will enable the reader to realize this dependence.

It emerges from a consideration of these diagrams that the limit of resolution in depth is given in each case by the extent to


A
B


b"
C b
b
a
b"
D b
b
a

which the receiving screen must be set forward or back to achieve the expansion of the focal point of the interpenetrating vista into a disc whose luminosity is sufficiently reduced to permit of the focal point of the antecedent or succeeding point vista standing out upon it in clear relief.

19. Surface vistas.

By the term surface vista we may, as was suggested above, denote the aggregate of point vistas, which, taking origin side by side upon one and the same optical plane, pass through the aperture of the same lens.

Configuration and structure.

We may distinguish in the surface vista :—

(*a*) A plane of origin—corresponding to the object field.

(*b*) An equatorial or apertural plane. This is variously designated the *Ramsden disc*, the *Lagrange disc*, or the *pupil of the lens*.

(*c*) A terminal plane—corresponding to the focussed image.

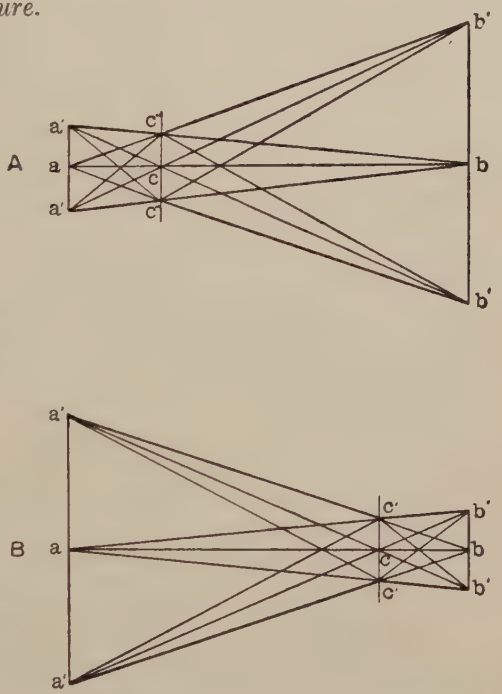


FIG. 30.

In the case of the surface vistas shown in Fig. 30, the plane of origin is in each case indicated by the letters a', a, a' ; the terminal plane by the letters b', b, b' ; the equatorial plane by the letters c', c, c' ; and in the case of the axial beam the object-aperture distance by a, c', and the aperture-image distance by the letters c', b.

20. Configuration of (*a*) the simple magnifying vista, of (*b*) the simple minifying vista, and of (*c*) the vista in which magnification is followed by minification.

The figure above brings before the eye the two more important varieties of surface vistas.

Magnifying vista. The distinguishing features of a magnifying vista (Fig. 30, A) are the following :—

(*a*) Its terminal plane is larger than its plane of origin ;

(*b*) Its closing limb is longer than its opening limb ;

(*c*) Its closing angle is smaller than its opening angle.

Minifying vista. A minifying vista (Fig. 30, B) can be recognized by three features :—

(*a*) Its terminal focal plane is smaller than its plane of origin ;

(*b*) Its closing limb is shorter than its opening limb ;

(*c*) Its closing angle is larger than its opening angle.

Magnifying and minifying vista. It will be convenient to discriminate between simple magnifying vista represented in B, and such vista as that represented in Fig. 63, where magnification obtained by the agency of the front lens is reduced by the agency of a supplementary lens. It will be recognized in the diagram that the supplementary lens bends in the diverging axes of the outlying point vistas, and thus diminishes the magnification, while it gives, by bringing the terminal poles of these point vistas into the field, a corresponding extension to the field of view.

21. Dimensions which come into consideration in the case of surface vistas.

The dimensions which we have to consider in the case of a surface vista are :—

(1) The diameters of the plane of origin and the terminal plane respectively ;

(2) The length of the opening and closing limbs ;

(3) The diameter of the apertural plane, and

(4) The sines of the opening and closing angles respectively, i.e. the ratios which obtain between the semi-diameter of the aperture and the length of the opening, or, as the case may be, of the closing limb.

The second, third, and fourth of these dimensions correspond in each case to those of the point vista inscribed about the optical axis.

22. Relation between the configuration of the surface vista and the scale of the image.

If we are furnished with the diameters of the object field and image field respectively, we obtain the magnification by dividing the first of these magnitudes into the second, in conformity with the formula :—

(a) $Magnification = \frac{Diameter\ of\ terminal\ plane\ of\ the\ vista.}{Diameter\ of\ the\ plane\ of\ origin\ of\ the\ vista.}$

If we are furnished with the lengths of the opening and closing limbs we can, in the case where vistas such as those in Fig. 25 *a* and *b* are in question, obtain the magnification by dividing the first of these magnitudes into the second in conformity with the formula :—

(b) $Magnification = \frac{Length\ of\ the\ closing\ limb\ of\ the\ vista.}{Length\ of\ the\ opening\ limb\ of\ the\ vista.}$

If we are furnished with the numerical aperture of the opening and closing limbs of the central point vista (N.A. and n.a. respectively) we arrive at the magnification by dividing the first of these measurements by the second, in accordance with the formula :—

(c) $Magnification = \frac{N.\ A.}{n.\ a.}$

23. Examples of the application of the formulae for the determination of the magnification of the image.

Example 1. The diameter of the receiving screen (terminal plane of the vista) is 1 cm. The diameter of the object field in view (plane of origin of the vista) is 1 mm.

Applying Formula (*a*), we find that the magnification is 10-fold.

Example 2. The focal distance at which the lens is working (length of the opening limb of the vista) is 1 cm.

The conjugate focal distance at which the image is formed (closing limb of the vista) is 10 cm.

Applying Formula (*b*), we find that the magnification is 10-fold.

Example 3. The Numerical Aperture of the opening angle (N.A.) is 0·2. The numerical aperture of the closing angle (n.a.) is 0·02.

Applying Formula (*c*), we find that the magnification is 10-fold.

24. Extent of the object field which is imaged.

In the case where the magnification and the diameter of the terminal plane have been determined, the dimensions of the object field which is included in the image are arrived at in the following simple manner :—

$$Diameter\ of\ the\ object\ field = \frac{Diameter\ of\ the\ image\ field.}{Magnification\ of\ the\ image.}$$

25. Examples of the application of the above formula.

Example 1. The diameter of the image field is 1 cm. The magnification of the image is 10-fold. The diameter of the object field in view is 1 cm. ÷ 10 = 1 mm.

Example 2. The diameter of the image field is, as before, 1 cm. ; the magnifying power is 50-fold ; the diameter of the object field in view is 1 cm. ÷ 50 = 0·2 mm.

26. Quality of the image.

The excellence of the image consists—

(*a*) In the achievement of separate definition in the image of radiant points which lie side by side upon one and the same optical plane.

(*b*) In the separate definition of radiant points lying one behind the other;

(*c*) In the satisfactory imaging of such radiant points as may be hidden from direct view by intervening obstacles.

27. Influence exerted by the aperture of the vista upon resolution in plan.

Inasmuch as the resolution of the image is affected by everything which causes the focussed light of the individual beam to be dispersed over a surface of appreciable extent instead of being concentrated upon a mathematical point, the full discussion of the question of resolution must necessarily be reserved till we have had an opportunity of considering the phenomena of spherical and chromatic aberration and of diffraction.

It will suffice for the moment to note with regard to impairment of resolution which is due to diffraction, that this acquires importance only in the case where light passes through a contracted aperture.

28. Influence exerted by the aperture of the vista upon resolution in depth.

We have already, in considering the quality of the image obtained in the point vista, seen that the image plane and in correspondence with this the plane of origin of the beam cannot be located with accuracy except in the case of a wide-angled vista. Again, we have seen in connexion with interpenetrating vistas, that the wider the aperture the more clearly does the focal image stand out from the diffusion discs of succeeding or preceding vistas.

In the case of aggregates of point vistas such as we are here dealing with, similar but proportionately greater advantages are obtained by the employment of the wide aperture. The advantages of increased aperture are more particularly sensible in the case where the element which is to be viewed lies immediately in front of another similar element.

Such a case is represented in Plate VI, C and D.

In each case a blue object point, *a*, lies directly in front of another blue point, *b*.

In D, where we are dealing with a narrow-angled vista, the point *b* is imaged on a field of saturated blue furnished by the beam which proceeds from *a*. As a result, the resolution of the points *a* and *b* is very imperfect.

In C, on the contrary—owing to the invasion of the centrally placed blue vista derived from *b* by the colourless flanking vistas derived from *b′*, *b″* —*b* is clearly imaged as a blue point upon a field of a pale unsaturated blue.

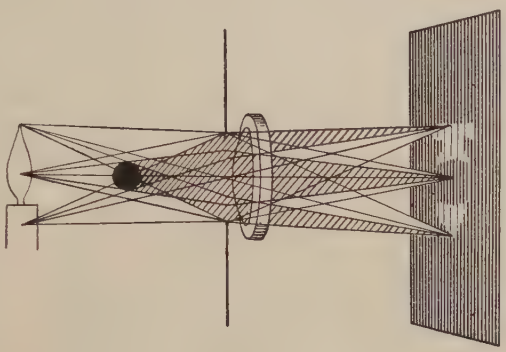


Fig. 31.

29. On the aperture of the vista as a factor which determines the extent of the plane of origin occulted by an intrusive element and the more or less satisfactory representation in the image of partially eclipsed elements.

We may most conveniently study the effect of the intrusion of obstacles into a surface vista by the aid of the following experiments.

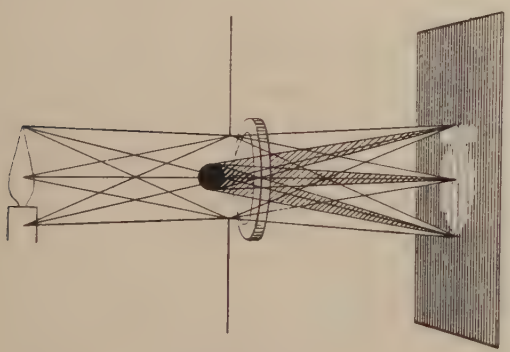


Fig. 32.

Experiment 1. Take a convex lens possessing a focal length of 4 to 6 inches, and, holding it at some 8 inches from a lighted candle, project a focussed image of the flame upon a screen. Now introduce into the surface vista thus constituted an obstructing obstacle, such as the point of a pencil.

Note the following points :—

(*a*) When the obstacle is introduced into the vista, either in the neighbourhood of its plane of origin or in the neighbourhood of its terminal plane, the shadow of the pencil will lie as a dark band across the image. In addition to the band of total eclipse, an area of penumbra will appear upon the image (Fig. 31).

(*b*) When the obstruction is brought closer to the lens the area of total eclipse will be diminished and the area of penumbra proportionately extended.

(*c*) When the point of the pencil is, as in Fig. 32, brought up close

to the lens, no portion of the candle undergoes total eclipse. The shadow will now be diffused in the form of a faint penumbra over the whole extent of the image.

Experiment 2. Repeat the experiment with the lens stopped down by placing it behind a small aperture in a sheet of cardboard.

The obstruction, wherever introduced, now produces a wider band of total eclipse in association with a more extensive penumbra.

It is to be noted that when the aperture in the cardboard is reduced to very small dimensions the lens image no longer possesses any sensible advantage over the image produced by the simple unarmed aperture.

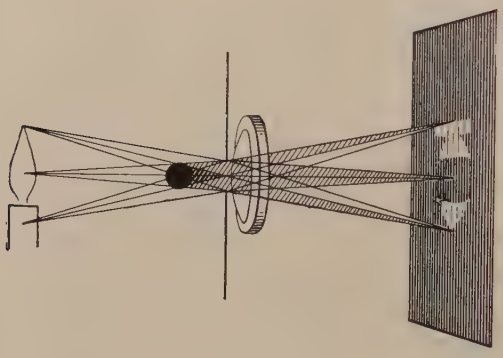


Fig. 33.

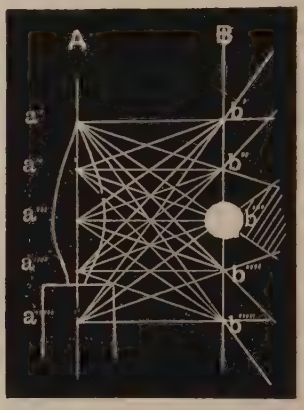


Fig. 34.

It has been brought out in the experiments above, and in the figures which illustrate them, that the intrusion of obstacles into a surface vista may have as its result, on the one hand, the development of areas of complete eclipse, and, on the other hand, the development of incomplete eclipse or penumbra.

It will be understood that a point of complete eclipse corresponds in each case to the complete occlusion of a beam ; a point of partial eclipse to a partial occlusion.

30. Conception of a vista as a sequence of radiant planes.

The data which have been furnished by the experiments set forth above can be analysed in a number of different ways.

(*a*) We may proceed upon the conception that we are dealing in the case of the candle and pencil with a surface vista which contains no other radiating points beyond those positioned in the candle flame.

(*b*) We may, on the other hand, conceive of our surface vista as containing not only the fundamental radiant plane of the candle, but also a continuous succession of derivative radiant planes.

Fig. 34, which reproduces with further detail the candle flame

and obstacle represented in Figs. 31, 32 and 33 *supra*, and in Fig. 35 *infra*, will aid in the realization of these two different points of view.

If we here regard the luminous points a', a'', a''', a'''', a''''', in the plane of the candle flame (A) as the sole sources of light, and if we leave out of account as sources of light the nodal points b', b'', b''', b'''', b''''', produced by the intersection of the rays from a', a'', a''', a'''', a''''' in the plane B, the obstructing object will be conceived of only as an opaque obstacle and the dark patch in the image on screen A′ in Fig. 35 only as the shadow and penumbra of that obstacle.

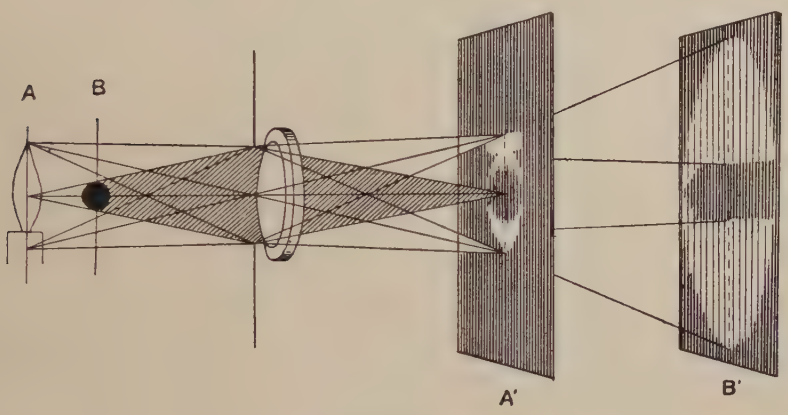


Fig. 35.

If on the other hand, we take the view that the nodal points b', b'', b''', b'''', b''''' are, equally with the radiant points in the candle flame, entitled to rank as light sources, then the obstructing object will come into consideration also as the original of the image in B′, and the shadow which lies upon the image on screen A′ (Fig. 35) will come under consideration as the forerunner of the focussed image which appears upon the screen B′.

The simplification which is achieved by viewing from this latter point of view the problems which arise in connexion with image formation in the microscope will appear as we proceed.

31. Conception of a catena of vistas.

When, instead of placing the receiving screen at the terminal plane of the vista we carry it farther away, the focussed beam will, as we have seen, spread out again behind the focal point. A further lens or combination of lenses may then be employed to re-focus these diverging rays.

A *catena of vistas* will now have been constituted.

In the figure below are figured different types of such catenas. At A we have a catena of three elements corresponding to a beam in the optical axis which is focussed by one lens, re-focussed by the second, and again re-focussed by a third lens.

At B we have a catena corresponding to an axial beam which is focussed in the first vista by two lenses and again re-focussed in the second vista by two lenses.

At C we have a catena corresponding to the path of an obliquely

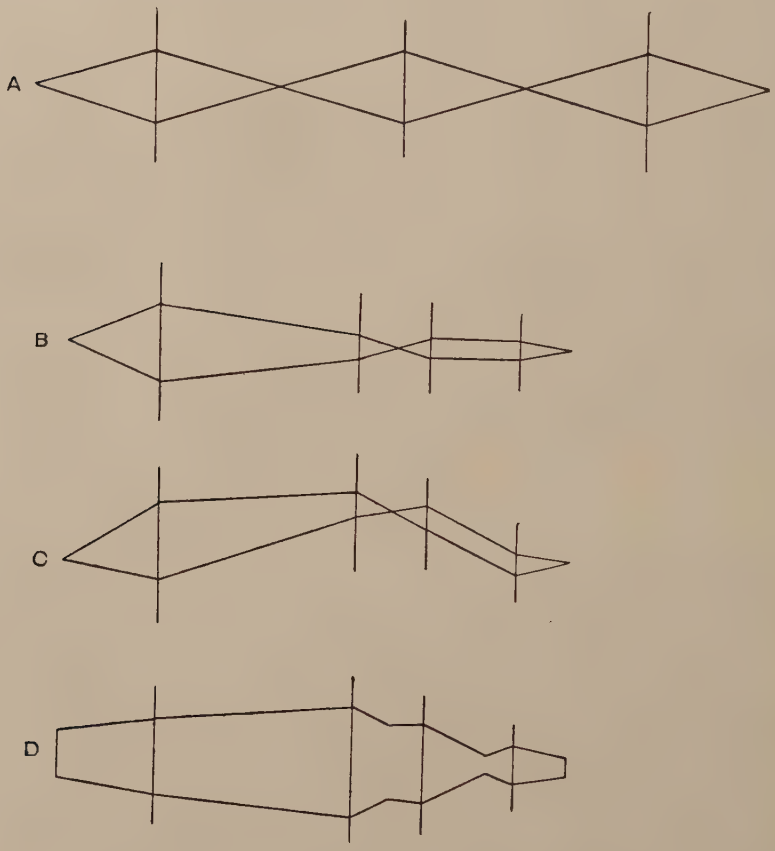


FIG. 36.

incident beam which is focussed and re-focussed by the same series of lenses.

At D we have the catena of surface vistas which represents the catena shown in B, flanked on either side by a catena similar to that shown in C.

32. Image formation in a catena of surface vistas.

It will have been appreciated that, when a system of beams has

been focussed upon the terminal plane of a first vista and has then been re-focussed, there is obtained upon the terminal plane of the second vista a replica of the first picture.

The replica obtained on the terminal plane of such a following vista is in no case a facsimile reproduction of the first image.

It differs from it—

(1) In the respect that the image has undergone inversion

An additional vista is often introduced into the composition of an optical instrument, as, for instance, in the case of the "day telescope," for the purpose of converting an inverted into an erect image.

(2) In the respect that the image is ordinarily an image on a different scale.

In point of fact, it is primarily for the achievement of cumulative magnification that concatenation of vistas is resorted to in optical instruments.

(3) In the respect that there is superadded to the original picture a focussed image of any adventitious object which is disposed in any one of the preceding image planes,

An additional vista may—as, for instance, in the case of the eikonometer, described below—be introduced into a catena for the purposes of applying a measuring scale to an image.

(4) In the respect that the beams come into focus in each succeeding vista at a different angle and yield, as we shall see, in conformity with this, a different diffraction picture.

33. Law governing the inversion of the image in a catena of surface vistas.

By the re-inversion of the inverted image formed upon the terminal plane of the first vista we obtain an erect image upon the terminal plane of the second vista. In the case where a third vista is employed we obtain again an inverted and, in the case where a fourth vista is added again an erect image. In brief, an inverted image is obtained with every odd, an erect image with every even number of concatenated vistas.

34. Methods of measuring the scale of the image obtained on the terminal plane of a catena of vistas.

As indicated above, the terminal image is ordinarily on a different scale from the image formed on the intermediate focal plane or planes. The magnification of the terminal image can be arrived at by a variety of different methods.

(1) We may measure by any of the methods which are appropriate for this purpose the magnification achieved in each component vista separately and then multiply together the figures we have obtained.

(2) We may, as is done, for instance, in the case of the eikonometer, presently to be described, apply a scale to the terminal image and compare the dimensions of this image directly with those of the original object picture.

(3) We may divide the N.A. of the opening angle by the n.a. of the closing angle of the catena.

The last-mentioned method may with advantage be elucidated, as it leads up to the consideration of some of the most important optical properties of a catena of vistas.

35. Determination of the scale of the terminal image of a catena of vistas by dividing the numerical aperture of the opening angle (N.A.) by the numerical aperture of the closing angle (n.a.).

The quotient furnished by the division of the numerical aperture of the opening angle of a vista by the numerical aperture of its closing angle furnishes, as we have seen in *subsecs.* 22 and 23 *supra*, the measure of the magnification achieved in the individual vista.

What holds true with regard to the appraisement of the magnification of the unconcatenated vista holds true also with respect to the appraisement of the cumulative magnification achieved in a catena of vistas.

This follows from the circumstance that the closing and opening angles of successive vistas of a catena are (as will be seen on referring to Fig. 36 A, B, and C, or to Fig. 37, which illustrates *Example* 1 below) in each case vertical and, by consequence, equal angles.

In accordance with this we arrive at the same arithmetical result when we divide the sine of the opening angle of an antecedent vista, by the sine of the opening angle of the next following vista, as when we divide the sine of any opening angle by the sine of the closing angle of the same vista.

Following this out upon Fig. 36, in connexion with the first example given below, it will be seen that the final result arrived at will be the same whether we—

(*a*) Divide directly the sine of the opening angle of the catena by the sine of its terminal angle, or whether we—

(*b*) In a series of operations—

(α) Divide the sine of the opening angle of the first vista by the sine of the closing angle of this vista,

(β) Divide the sine of the opening angle of the second vista by the sine of its closing angle,

(γ) Divide the sine of the opening angle of the terminal vista by the sine of its closing angle, and finally,

(δ) Multiply together the quotients obtained in this series of operations.

Example 1.—Required the magnification achieved in a catena of vistas whose dimensions are as in the diagram and table below.

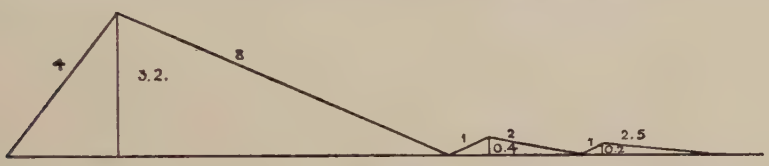


FIG. 37.

Serial Number of Vista.	Measurement of Vistas. 1 Length of opening limb.	2 Length of closing limb.	3 Semi-diameter of the apertural plane.	4 Angular aperture (sine) of opening angle.	5 Angular aperture (sine) of closing angle.	Magnification achieved in the separate vistas as deduced from the ratio between measurements 1 and 2; or alternatively between measurements 5 & 4.
Vista No. 1	4	8	3·2	$\frac{3\cdot2}{4}=0\cdot8$	$\frac{3\cdot2}{8}=0\cdot4$	2
„ „ 2	1	2	0·4	$\frac{0\cdot4}{1}=0\cdot4$	$\frac{0\cdot4}{2}=0\cdot2$	2
„ „ 3	1	2·5	0·2	$\frac{0\cdot2}{1}=0\cdot2$	$\frac{0\cdot2}{2\cdot5}=0\cdot08$	2·5

The cumulative magnification is obtained indifferently by multiplying together the quotients representing the magnification of the separate vistas $2 \times 2 \times 2\cdot5$ (*vide* last column of Table), or by dividing the sine of the opening angle of the system (0·8) by the sine of the terminal angle of the system (0·08).

The magnification works out by either procedure as a 10-fold magnification.

Example 2. Required the magnifying power of the same catena of vistas, in the case where the opening limb of the system is measured in oil possessing a refractive index of 1·5, the other distances being, as before, measured in air.

Instead of dealing, in the case of the opening angle, simply with the sine, we have to deal with the N.A., i.e. with the sine of the opening angle multiplied by the refractive index of the oil in which the focal length is measured.

Substituting for the simple angular aperture 0·8, given in column 4 of the Table above, the numerical aperture (0·8 × 1·5 =)1·2, and leaving the measurements of the other angles, as given in column 5, unaltered, we obtain here a magnification of (1·2 ÷ 0·2 =)6 for the two first vistas taken in conjunction, and a magnification of (1·2 ÷ 0·08 =)15 for the whole system.

36. On the imaging of the successive optical planes which are constituent elements of the original vista.

A good deal more than has as yet appeared is involved in the concatenation of vistas. When we concatenate one vista to another we reproduce in the following vista, not merely the image of the plane of origin of the first vista, but also images of the succession of radiant planes which together constitute that first vista.

Put otherwise, every following vista in a catena is built up of a series of replicas of the successive optical planes which constitute the antecedent vista.

The scale and general character of these replicas taken severally is determined by the operation of the self-same laws which have application to the reproduction of the image of the plane of origin of the catena.

(*a*) The successive images are in each case inverted.

(*b*) Again within certain limitations the scale of the images is determined by the relative lengths of the object-aperture and aperture-image distances which come in each case into consideration.

(*c*) Adventitious elements which are included anywhere in an antecedent vista are reproduced in accurate focus upon the corresponding optical plane in the following vista.

The general principle having been laid down, the case of the imaging in successive vistas of the apertural plane of an antecedent vista suggests itself for special consideration.

37. Imaging of the apertural plane of an antecedent vista in the apertural planes of all the following vistas.

As a preliminary to considering the imaging of the apertural plane of an antecedent vista in the following vistas of a catena, we shall do well to realize, that the apertural plane of a surface vista which is generally conceived of only as a blank field traversed by a system of intersecting pencils of parallel rays, may also, as will be seen on referring to Plate VII, Figs. 3 and 4, be conceived

of as an object plane occupied by radiant points corresponding to the points of intersection of the rays which pass through the aperture.

Each of these ways of conceiving of the apertural plane is inadequate. We achieve an adequate conception only when we realize that we have to deal in the aperture of every lens in a catena with two systems of luminous impulses. One of these categories of luminous impulses has in the aperture reached what we speak of as the mid-point in its journey from plane of origin to plane of focal destination. These impulses are those we have in view when we think of the apertural plane as occupied by pencils of parallel rays.

From the nodal points formed by the intersection of the rays of these several pencils there springs into birth a new system of luminous impulses. The birthplace of this new system is in the apertural plane of the lens.

When now the luminous impulses first mentioned come to focus upon the terminal plane of the first vista, forming thereon an image of their plane of origin, those luminous impulses, just referred to, which radiate from the nodal points in the apertural plane, will, in conformity with the fact that they are exactly half the length of a vista behindhand, have arrived only at that phase of their journey in which they, as pencils of parallel rays, reciprocally interpenetrate.

These luminous impulses will arrive at their plane of focal destination half the length of a vista farther on, that is to say, in the apertural plane of the second vista of the catena, and they will there furnish a focussed image of the apertural plane of the first vista.

The same sequence of events will repeat itself in every vista. Of the two systems of luminous impulses, the antecedent one will always attain its focal phase when the following one attains its Ramsden disc phase; and, vice versâ, the following one will always attain its focal phase where the antecedent one attains its Ramsden disc phase.

The plane of origin and the original apertural plane will, as a result, be imaged and re-imaged in an alternating manner throughout the whole length of the catena.

As a corollary to the above, we may note that the sectional area which functions, in relation to the system of impulses which derive from the plane of origin of the catena, as image field functions, in relation to the system of impulses which derive from the apertural plane, as Ramsden disc; and, vice versâ, the sectional area which functions in relation with the first-mentioned system of impulses as

Ramsden disc, functions in relation with the system of impulses which derive from the apertural plane as image field.

38. Preliminary indications of the practical applications of the fact that in a catena the field upon which is imaged the apertural plane of the antecedent vista corresponds in area and position with the apertural plane of the following vista.

The circumstance that the image of the original aperture is carried on in a catena from vista to vista, and that it is positioned in each vista in the Ramsden disc of that vista, occupying the same sectional area, can be turned to account in two ways.

It enables us, on the one hand, to obtain the sectional measurements required for the determination of the sines of the opening and closing angles of a catena. It enables us, on the other hand, to scrutinize the aperture of the first, or any following, vista in the aperture of any succeeding vista.

39. Exploitation of the circumstance that the sectional measurement of the image of the aperture of the antecedent vista furnishes the sectional measurement of the Ramsden disc of the following vista.

The sectional measurement of the Ramsden disc of the terminal vista which is required in connexion with the determination of the cumulative magnification of the catena can, in the case of the microscope, be obtained by applying a scale to the image of the objective aperture which is formed on a plane a little above the eye-lens.

Procedure. Take in one hand a pocket lens and in the other a finely divided transparent scale, such as is available in the diffraction grating (ruled with 16 lines to the millimetre) supplied in the cover of this book. Bend down over the eye-piece until the aerial image of the back surface of the objective (Plate X, Figs. 1, 2, 3), which is positioned at a distance of perhaps 5 mm. to 10 mm. above the eye-lens, is brought into view. Keeping this under observation, bring the measuring scale accurately into the same optical plane, and read off upon it the number of divisions covered by the image. The measurement thus read off corresponds to the diameter of the Ramsden disc of the terminal vista of microscope.

From the measurement made as above the diameter of the aperture of the opening vista of the microscope is obtained by multiplying by a factor corresponding to the magnifying power of the ocular (*vide Cap.* XIII, *subsect.* 45).

40. Exploitation of the circumstance that in a catena the aperture of every antecedent vista is imaged and available for scrutiny in the Ramsden disc of the terminal vista.

The following are some of the ways in which we can, in connexion with microscopic work, turn to account the fact that the whole sequence of apertures through which the light passes in the microscope can be found imaged in the Ramsden disc of the eye-lens.

We can, by examining the Ramsden disc of the eye lens by the aid of a magnifying glass, ascertain—

(*a*) Whether the aperture of the objective is or is not fully filled in with the transmitted beam ;

(*b*) Whether the back lens of the objective is encumbered with dust ;

(*c*) Whether the front lens of the objective—in the case where this is an immersion lens—is encumbered with an air bubble ;

(*d*) Whether the substage diaphragm is of suitable dimensions ;

(*e*) Whether the centre of the substage diaphragm is in accurate alignment with the centre of the aperture of the objective.

41. Association of progressive restriction of the aperture with cumulative magnification, and preliminary indications of the significance of this progressive restriction of the aperture in connexion with the development of a critical image, and the limit of microscopic resolution.

We have seen that the angular aperture of the closing limb is less than that of the opening limb in every magnifying vista.

We have further seen in the course of working out the examples in *subsect.* 35 *supra*, that in a catena in which cumulative magnification is achieved, the Ramsden disc, and in association with this the angular aperture of the terminal beam, diminishes in a progressive manner in each successive vista.

This restriction in the diameter of the transmitted beam entails the following consequences which are of absolutely fundamental importance in connexion with the microscopic image.

(1) As the diameter of the beam becomes more and more restricted the disturbance which is due to diffraction is rendered more and more prominent.

This disturbance will come up for consideration in *Cap.* IX.

(2) The difficulty of focussing the eye in an accurate manner upon the image is increased.

Vide supra, Fig. 28, and *infra*, *Cap.* X, *subsect.* 9, *Experiments* 2 and 3.

(3) As the diameter of the beam diminishes, smaller and smaller intrusive obstacles—and we can never get away from

small obstacles in the form of dust upon the eye lens and eyelashes and intra-ocular opacities—eclipse more and more of the image. Finally the retinal screen is so encumbered with shadows that distinct vision is at an end.

In point of fact, it is in the last instance, as the reader will see on referring to *Cap.* XVI and Plate XVIII, the narrowing of the beam and the projection of shadows upon the retina which imposes a limit upon the magnification used in the microscope.

CHAPTER VIII.

ON THE DEFECTS WHICH ARE INTRODUCED INTO THE IMAGE BY SPHERICAL AND CHROMATIC ABERRATION IN THE LENS.

Introductory—Distortion by spherical aberration—Method of correcting the distortion produced by spherical aberration—Distortion due to chromatic aberration—Method of correcting for chromatic aberration.

1. Introductory.

In the foregoing chapter it has been tacitly assumed that a beam taking origin from a mathematical point in the object is, by the agency of the lens, brought to focus upon a mathematical point in the image. It was in like manner assumed that beams taking origin upon one and the same optical plane in the object would in the image be brought to focus at one and the same distance behind the lens.

In point of fact, neither the one nor the other of these assumptions holds true. The lens in every case produces distortion. We may discriminate between a *distortion by spherical aberration* and *distortion by chromatic aberration.*

2. Distortion by spherical aberration.

Where a lens which has not been corrected for spherical aberration is employed the transmitted beam is shaped, not into a cone converging in a regular manner upon a single focal point, but into a *caustic* converging as shown in the diagram (Fig. 38, A) upon a series of focal points disposed one behind the other upon the same axis. It will be seen that the most remote of these focal points corresponds to the rays which traverse the centre of the lens, and that the nearest corresponds to the rays which traverse the periphery of the lens. As a result of this the image of a radiant point is constituted not by a single mathematical point, but by a focal point enveloped in a diffusion disc.

Again, in the case where the lens is uncorrected for spherical aberration, beams proceeding from different parts of the field are brought to focus upon different focal planes. The beams which take origin in the centre of the field converge upon a more remote, beams from the periphery of the field upon a nearer focal plane.

Fig. 38.

As a result, a plane surface in the object is in the image represented by a surface which is concave towards the lens. And as a further result, elements in the centre of the field of the image are, in conformity with the fact that the corresponding beams come to focus upon a remoter focal plane, more highly magnified than those in the periphery of the image field (Fig. 38, B).

Both these forms of distortion become progressively more pronounced as the convexity of the lens increases.

Experiment 1. Take a capillary filament of glass and shape it into a spherical lens by fusing its extremity in the flame as already explained (*supra*, *Cap.* II, *sect.* 1, *subsect.* 2).

Hold this lens at some little distance from this page in such a manner as to furnish a minified image of the print. Examine this image through a pocket lens, and note that the letters which occupy the centre of the field of view are imaged on a much larger scale than the letters which occupy the periphery of the field (Fig. 38, B).

Experiment 2. View through the lens a system of parallel lines. Note that they are distorted in the manner shown in Fig. 39, A.

Experiment 3. Taking up a position within a few feet of the window and facing it, hold up the spherical lens before one eye and note that the window is represented in the image by a barrel-shaped figure (Fig. 39, B). This barrel-shaped configuration is conditioned by the circumstance that the panes in the centre of the field of view are represented in the image on a larger scale than the panes in the periphery of the field.

Experiment 4. Inscribe on the window pane with a glass-writing pencil a system of parallel lines or a ruling of squares and set up in front of it a microscope provided with an Abbe or another uncorrected condenser. Focus down now with a one-inch objective upon the image of the ruling which is furnished by the condenser. Note that the parallel lines or, as the case may be, the squares are in the image distorted in exactly the same manner as the lines in *Experiment* 2 or the panes in *Experiment* 3, *supra*.

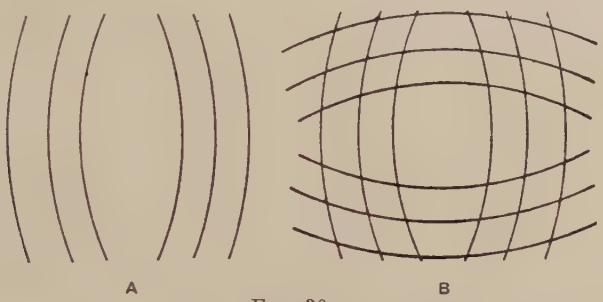


Fig. 39.

SHOWING THE NATURE OF THE DISTORTION WHICH IS EFFECTED BY SPHERICAL ABERRATION.

3. Method of correcting the distortion produced by spherical aberration.

Spherical aberration is corrected by lengthening out the focus in the case of those rays which traverse the peripheral zones of the lens. This is achieved by combining concave with convex lenses and also in a subsidiary manner by re-adjusting, as occasion may require,the relative positions of the lenses in any optical combination.

The last-mentioned method of correcting spherical aberration is placed at the disposal of the microscopist, in the case where the objective is fitted with a correction collar (*vide infra, Cap.* XIII, *subsect.* 36, under Correction Collar).

A lens system which has been corrected for spherical aberration is spoken of as an *aplanatic* combination—the term aplanatic finding its appropriateness in the circumstance that in the case where the lens is duly corrected there is no longer any wandering away of the component rays of the transmitted beam from the conjugate focal point.

The following points are to be observed—

(1) All that it is practicable to achieve in the matter of the correction of the spherical aberration of a lens is to achieve an undistorted image upon some one selected conjugate focal plane.

We shall see hereafter, in connexion with the objective and draw tube (*Cap.* XIII, *subsects.* 35 and 42), and again (*Cap.* XV, *subsect.* 10),

in connexion with the achievement of a critical microscopic image that in the case of objectives designed to work respectively with the long, 250 mm. "English," or short, 160 mm. "Continental" tube, the critical image is obtained only when the tube length is adjusted exactly to these standard focal lengths.

(2) A lens system may be designedly over- or under-corrected by the maker.

We shall see hereafter (*Cap.* XIII, *subsect.* 35, *infra*) that dry objectives are habitually under-corrected in order to make provision for the distortion of the opening limb of the beam, which is effected by refraction where the beam emerges from the cover-glass into air.

The curvature of the field, which has been enumerated among the distortion effects produced by spherical aberration, is not abolished by the corrections which are introduced for the purpose of rendering the lens aplanatic.

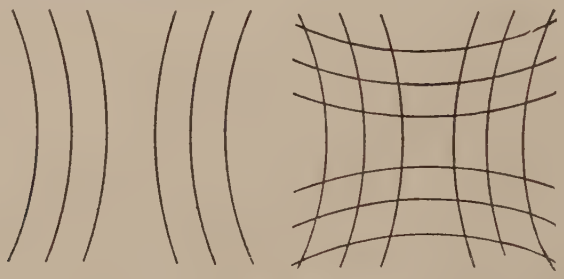


Fig. 40.

SHOWING THE NATURE OF THE DISTORTION WHICH IS ASSOCIATED WITH THE OVER-CORRECTION OF THE SPHERICAL ABERRATION OF A LENS.

4. Distortion due to chromatic aberration.

In view of their different refrangibility, the different components of white light are in the case where they traverse an uncorrected lens, brought to focus on different focal planes. In particular, the more refrangible blue rays are brought to focus on a nearer, the less refrangible red light on a plane which is more remote from the lens (Plate VII, Fig. 1).

The effect of this chromatic aberration upon the image is two-fold—

(1) The image of every radiant point is encompassed by coloured fringes corresponding to the diffusion discs of those components of the beam which focus, as the case may be, upon a nearer or a farther focal plane than that which happens for the moment to be under examination.

Plate VII.

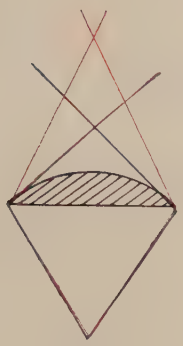


Fig. 1.

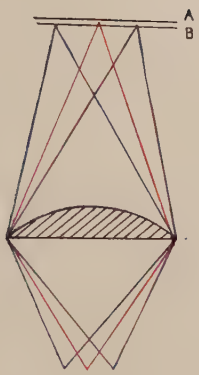


Fig. 2.

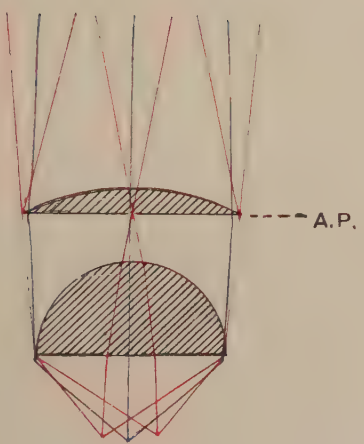


Fig. 3.

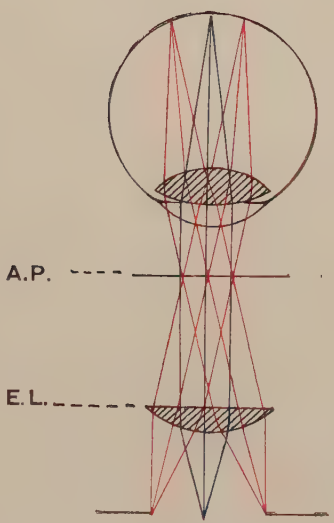


Fig. 4.

BATTELLE
PUBLIC
LIBRARY

These coloured fringes are developed in the most conspicuous way along the edges of any shadow which happens to be projected upon the field of view. They can be seen also round the margins of the aperture of an uncorrected lens system.

(2) Points on the same radiant plane which emit light of different colour—for instance—as in Plate II, *d*,—blue and red light respectively,—are imaged on different conjugate focal planes and on a different scale. The blue objects will be imaged on a nearer plane and on a smaller scale ; red objects on a farther plane and on a larger scale (Plate VII, Fig. 2).

Experiment 1. Set up, as in *Experiment* 4, *subsect.* 2 *supra*, in front of the window a microscope provided with an Abbe condenser, and bring into view under a 1-inch or higher objective the image of the window bar which is furnished by the condenser. This will be seen fringed (as shown in Plate II *a* and Plate III *d*) with coloured bands.

Experiment 2. Place upon the stage of the microscope a film preparation of tubercle bacilli mixed with other bacteria and stained differentially as shown in Plate II *d*.

If the objective has not been properly corrected for chromatic aberration it will be found that the bacteria which are coloured in red and blue respectively are imaged, not as in the figure, on one and the same focal plane, but the bacteria which are stained red on a sensibly higher plane than the bacteria which are coloured blue.

Experiment 3. Hold up to the eye an ordinary high-power ocular. Note that the aperture of the diaphragm which represents the aperture of the combination is margined by a faint blue band.

5. Method of correcting for chromatic aberration.

While chromatic aberration can be abolished only by the employment of monochromatic light, more or less complete corrections can be effected by combining convex and concave lenses of different refractive indices.

What is aimed at in the case of the ordinary—so-called *achromatic objective*—is a bringing together of the foci of the red and blue rays, in particular, a bringing together of these foci in the case of the beams which occupy the centre of the field.

In the case of *apochromatic objectives* of Zeiss, a higher degree of chromatic correction is attained. Here the maker aims not only at the complete synthesis of the foci of the blue and red rays which lie at opposite ends of the spectrum, but also complete synthesis of the foci of the rays which occupy an intermediate position in the spectrum, and further he aims at the achievement of this correction over the whole area of the field.

For the attainment of this object it has been found necessary to over-compensate the objective and to under-compensate the ocular. The under-corrected oculars which complete the chromatic corrections of the apochromatic objectives are technically known as *compensation-oculars.* In the case of such under-compensated oculars the aperture of the combination which is brought into view by looking into the dismounted ocular in the direction of the window is fringed by an orange-coloured in lieu of, as in the ordinary ocular, by a blue band.

CHAPTER IX.

ON THE DEFECTS INTRODUCED INTO THE IMAGE BY DIFFRACTION OCCURRING IN THE APERTURE OF THE LENS.

Introductory—Brief exposition of the undulation theory of light—Conception of the vista modified in such a manner as to bring it into accord with the more adequate conception of the light distribution in the lens image now achieved, and signification to be attached to the term "antipoint"—Antipoint of a luminous point viewed through a restricted circular opening—Antipoint of a luminous point viewed through an aperture which is restricted along one diameter—Antipoint pattern of a file of points—Antipoint pattern of a luminous surface—Dependence of the antipoint pattern upon the brilliancy of the source of light, and distinction between "theoretical" and "conspicuous" antipoint. Law which governs the dimensions of the "theoretical," as distinguished from the "conspicuous" antipoint—Practical importance of the antipoint in connexion with the microscopic image—On the central elements of the antipoint and on the loss of resolution entailed by the overlapping of the false discs of adjoining antipoints—On the outlying elements of the antipoint considered in relation to the development of "spurious detail" in the image—Development of "intercostal spots"—Antipoint patterns furnished by diffraction gratings—Preliminary experiments with the diffraction grating—Dimensions of the antipoint pattern furnished by a grating—Examples, and verification of the formula for the dimensions of the antipoint furnished by a grating—Development of "intercostal lines" in such a form as to produce doubling or trebling in a periodical ruling.

APPENDIX I.—*On Abbe's theory of microscopic vision and on the effect of diffraction occurring in the object plane and opening limb of the vista.*

APPENDIX II.—*On the development of the median intercostal line considered as a fiduciary phenomenon upon which accurate adjustment may be made in making optical measurements.*

1. Introductory.

In the foregoing chapter we considered the effect produced upon the image by spherical and chromatic aberration, and we saw that it was possible by the aid of corrections to bring the different components of the beam to one and the same focus.

In this chapter we are confronted with the fact that there remain after the impediments to resolution which are furnished by spherical and chromatic aberration have been overcome, a further impediment.

The impediment in question is furnished by the diffracted light which comes off from the beam as it passes the aperture of the lens.

It will be advisable as a preliminary to describing and illustrating the effects produced by such diffraction to consider briefly the undulation theory of light which furnishes an explanation of these effects.

2. Brief exposition of the undulation theory of light.

The postulates of the undulation theory of light are the following :—

(*a*) Every luminous impulse is propagated outwards in all directions from its point of origin in the form of an expanding hollow sphere.

(*b*) Every point upon the radiant surface of this expanding sphere (this surface is technically denoted the *wave-front*) is a point of origin of a new luminiferous disturbance which is in its turn propagated through space in precisely the same manner as the original disturbance.

(*c*) The impulse is propagated by the communication to the particles of ether in each case of a movement transverse to the axis of propagation of the luminiferous disturbance.

(*d*) The transverse movement in question thrills at one and the same instant of time through the whole wave-front.

Otherwise expressed, the particles of ether included in the same wave-front are always in one and the same *phase* of movement.

(*e*) The direction of the thrill-movement is continuously altering, the movement being reversed every time that the wave-front has passed forwards through a distance corresponding to half a wave-length. It follows that, as the luminous impulse moves forward, its phase will correspond to, or be the reverse of, what it was at its point of departure, according as the path which has been traversed can be expressed in complete wave-lengths, or in odd multiples of a half wave-length.

(*f*) A sensation of light is originated whenever, under the influence of a succession of wave-fronts arriving with regular periodicity upon a sentient point on the retina, there is set up there by the lateral impact of the transversely vibrating particles of ether a sufficient succession of properly timed thrills.

Two altogether fundamental modifications are introduced into our every-day conceptions by the supersession of the emissory theory of light, and of the geometrical optics which have served us up to this point, by the conception of the luminous impulse as developed above.

We are in the first place compelled to conceive of every point on the retinal (or other) receiving screen as a point of impact for impulses emanating from every point of the radiant wave-front which occupies the aperture of the lens. Up to the present we have tacitly assumed that, in the case where an isolated beam is transmitted through a duly corrected lens, light would fall only upon the focal point, while the rest of the retina would remain dark.

In the next place, we can no longer, as we have hitherto, take it as assured that two or more rays—we may still conveniently employ the term ray to denote a system of impulses travelling along a line—will reinforce each other at their common point of impact. Nor even can we, as hitherto, take it as assured that such a point of impact will be a luminous point. Instead of this, we must conceive of the common point of impact of a number of rays merely as a point of convergence for impulses which will, according as their respective thrills tend on the whole to reinforce or to neutralize each other, call into existence, or fail to call into existence, a sensation of light or, as the case may be, some other light effect.

We may consider, in the first place, what will be the result of the arrival upon one and the same point of luminous impulses from two opposite points on the extreme confines of a wave-front.

(*a*) Where these points are so positioned that the paths from each to the point of impact are equal each to each, or, as is assumed to be the case in Fig. 41, A, optically equivalent, there will, owing to the circumstance that the impulses originating in these points will arrive in the same phase, be a simple arithmetical summation of the light effect.

(*b*) The same thing will hold true when the paths traversed differ by a whole wave-length as in D, or by any multiple of a wave-length.

(*c*) Where, on the contrary, the points of origin of the luminiferous disturbances are so positioned with relation to the point of impact that the paths differ by half a wave-length, as in C, or by any odd multiple of this, the succession of thrills conveyed from the one point will be exactly neutralized by a corresponding but antagonistic succession of thrills from the other. As the result of this antagonism, the light effect, which would have been evoked by either system of thrills operating separately, will be suppressed.

(*d*) Where the difference of path amounts to less than half as in B, or more than half and less than one complete wave-length, or to any multiple of such length, there will be at the point of impact a corresponding difference of phase between the impulses received.

A partial summation—or, if we choose to put it so, a partial extinction of the light effect—will in this case be effected.

Passing to consider the effect exerted upon the receiving screen

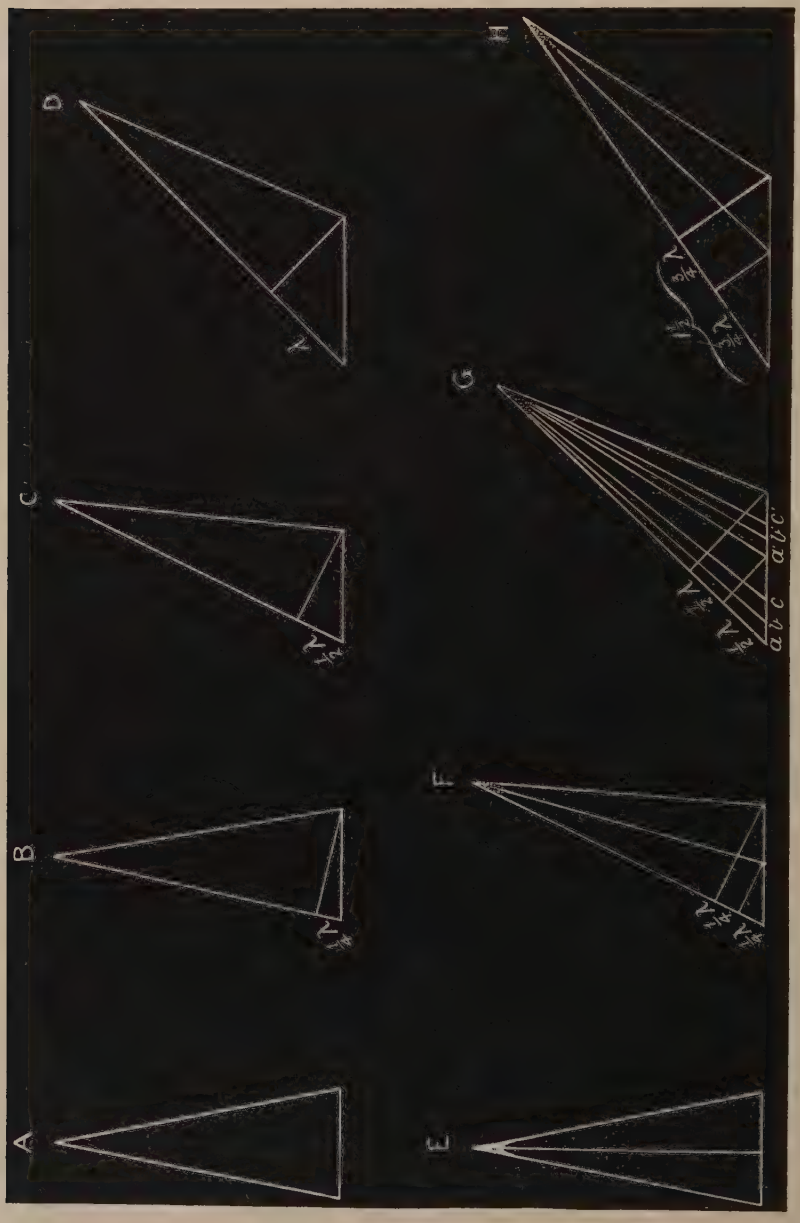


Fig 41.

by the sum of the impulses derived from the whole breadth of the wave-front intervening between A and B, we recognize that :—

(*e*) Where the point of impact is so situated that the paths from

every point on the wave-front to the point of impact are all equal, or, as is assumed to be the case in E, optically equivalent—and obviously this can occur only in the case where the point of impact is the focal point—there will be a simple summation of the light effects.

(*f*) Where the difference between the paths traversed by the light impulses from the extreme margins of the wave-front to the point of impact amounts, as in G, to a complete wave-length, the path of the impulse *a*, which starts at the margin of the wave-front, will exceed by half a wave-length the path of the impulse *a'* which starts from the centre of the aperture. There will be a similar difference between the path of the impulse which starts from the adjoining point *b* and that of the impulse which starts from the corresponding point *b'*. And the same difference of half a wave-length will obviously obtain as between the impulse from *c* and *c'*, and so on through the two halves of the wave-front. As a result the two halves of the wave-front will exactly neutralize each other, and the particular point of impact which, in the case considered above (Fig. 41, D), was a maximum point will here be a dark or minimum point.

The beam which converges upon such a dark point may be conveniently denoted a *caliginiferous beam*, i.e. a beam which brings darkness.

(*g*) In the case where the difference of path traversed by the impulses from opposite edges of the aperture amounts, as in F, to half a wave-length only, or to one and a half wave-lengths, as in H, or to any odd multiple of half a wave-length, the paths traversed by impulses from the corresponding points in the two halves of the wave-front will differ by one quarter of a wave-length, or by an odd multiple of this. Arriving, as they will, at the point of impact with a corresponding difference of phase, they will, as in the case considered above under (*d*), to some extent reinforce each the other, giving origin to an outlying maximum point.

3. Conception of the vista modified in such a manner as to bring it into accord with the more adequate conception of the light distribution in the lens image now achieved, and signification to be attached to the term "antipoint."

The conception of the point vista which has done duty up to the present must manifestly undergo some modification in order to bring it into accord with the more adequate conception which we have now formed of the distribution of the light in the lens image. For the conception of a vista converging by a closing limb upon a

single point of impact, a conception of a vista terminating, somewhat as indicated in Fig. 1, Plate VII, in a central closing limb converging on the focal point and in a number of outlying closing limbs converging on points disposed concentrically round the focal point.

The central element in this sheaf of terminal beams may now be distinguished as the *dioptric beam*, or as the *dioptric closing limb*, signifying by the term that the axis upon which this beam is centred is carried through the aperture from the pole of origin of the vista to the focal point. The outlying elements we may distinguish as *diffracted beams*, or as *diffracted closing limbs*, signifying by the term diffracted that the axes of the beams in question break off from, and form an angle with, the principal or dioptric axis of the beam.

We may further, in the case where we desire to discriminate the element in the image which is contributed by the dioptric beam from the element which is contributed by the diffracted beams, employ, in speaking of the first, the term, the *dioptric image*, and in speaking of the second, the term, the *diffraction image* or *diffraction pattern*.

Where, as will far more often be the case, we have to consider the aggregate image, we may, following Mr. Gordon's suggestion, speak of this as the *antipoint image*, or simply as the *antipoint*, designating by this term the full pattern of light which represents in the image a radiant point in the object.

4. Antipoint of a luminous point viewed through a restricted circular opening.

The antipoint of a point will manifestly be the pattern which is obtained by intercepting upon a receiving screen placed at the focus such a point vista as is represented in Plate VIII, Fig. 1, C.

In the pattern thus obtained the following elements are to be distinguished :—

(*a*) The central disk—technically, but infelicitously, spoken of as the *false disc*.

This element corresponds to the dioptric beam and to the adjoining diffracted beams, i.e. to those diffracted beams whose delimiting rays differ in length by less than one complete wave-length.

The brightness of this central disc is, as will be manifest, greatest at the centre where the dioptric beam impinges. It falls off rapidly towards the edge by virtue of the fact that the beams which here impinge become less and less luminiferous as the difference between their delimiting rays approaches to one whole wave-length.

Plate VIII.

Fig. 1.

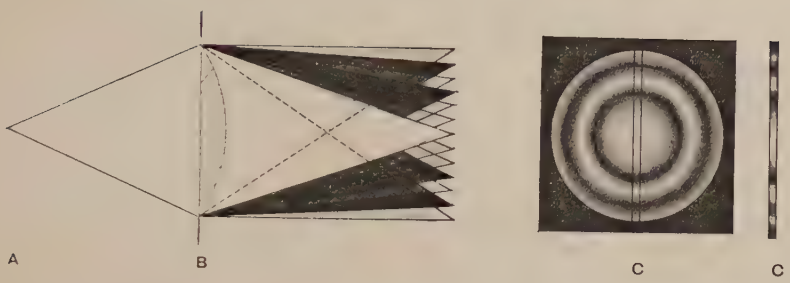


Fig. 2.

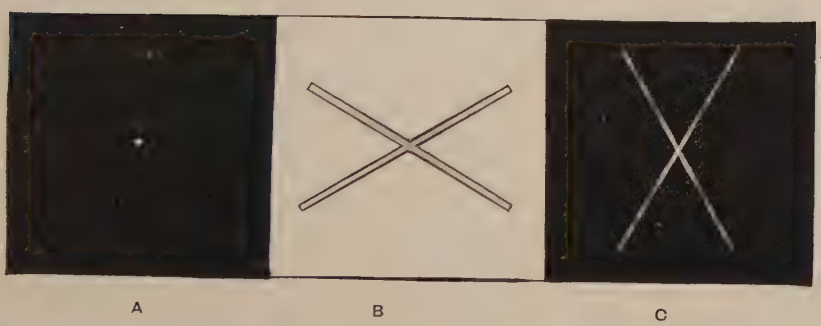
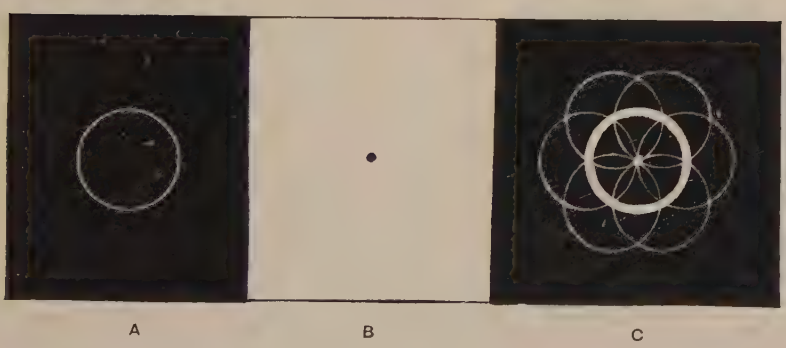


Fig. 3.



(*b*) A dark ring, corresponding to the points of impact of the caliginiferous beams, i.e. of the beams whose delimiting rays differ in each case by one whole wave-length.

(*c*) A bright enveloping ring, corresponding to the diffracted beams whose delimiting rays differ by more than one and less than two wave-lengths.

This element is, as will be seen, much inferior in brilliancy to the central disc.

(*d*) and (*e*) A further dark ring, corresponding to the caliginiferous beams whose delimiting rays differ by two whole wave-lengths, and a further bright ring, corresponding to luminiferous beams, whose delimiting rays differ by more than two and less than three whole wave-lengths.

Experiment. Place a very small globule of mercury upon any smooth surface and break it up into a number of almost microscopic globules by pressing down upon it a glass slip. Then dispose the globules in direct sunlight and view one of these from a distance of a few inches through a pin-hole in a card placed immediately in front of the eye. An antipoint which will correspond in all respects to that figured in Fig. 1, C, on Plate VIII, will come into view.

5. Antipoint of a luminous point viewed through an aperture which is restricted along one diameter.

The extreme case of an aperture restricted along one diameter is represented by a long narrow slit opening. When a luminous point is viewed through such an aperture the pattern obtained will be that set forth at Plate VIII, Fig. 1, C′. It will be recognized that this antipoint represents a median section of the antipoint in Plate VIII, Fig. 2, C, the central dash corresponding to the section of the central disc and the outlying points to the cross sections of the enveloping diffraction rings. It will be recognized on undertaking the experiments below, that the long diameter of the antipoint lies always at right angles to the long diameter of the slit opening.

Experiment 1. Rule a line with the point of a fine needle on a slip of glass which has been coated with soot in a candle flame. Turning the unsmoked side of the slip towards you, bring the slit opening, which is furnished by the scratch quite close up to the pupil, and view through it any bright point, such as that afforded by a distant candle flame. An antipoint similar to that shown in Plate VIII, Fig. 1, C′, will come into view.

Rotate the glass slip and verify that the long axis of the antipoint is in every case disposed at right angles to the direction of the ruling.

Experiment 2. Inscribe upon the smoked glass a second ruling intersecting with the first in such a manner as to make, let us say, the vertical angles formed by the intersecting lines acute and the horizontal angles obtuse. (Plate VIII, Fig, 2, B.)

View the distant candle through the point of intersection of the lines. There will now be brought into view, not the image of the diffracting aperture, but two median sections of the antipoint figure which will have been furnished by a point aperture. Observe that these optical sections are disposed in such a manner as to make the vertical angles obtuse and the horizontal angles acute. (Plate VIII, Fig. 2, C.)

6. Antipoint pattern of a file of points.

From the study of the antipoint figure of a point, we pass by a natural transition to consider the composite antipoint figure which corresponds to a line or file of points. Such a line, when seen through a slit opening, is represented in the image as shown in Plate IX, Fig. 1, by a broad principal line disposed between two less luminous flanking lines.

Where a small circular aperture is substituted for the slit, we have brought into view an antipoint pattern which, while it is made up of different constituent antipoints—to wit, of false discs and circles in lieu of dashes and dots—is in its essential features similar to the antipoint pattern furnished by the slit. This similarity is due to the fact that the rings of the constituent antipoints drop out of view, except in those regions where they intersect and reciprocally reinforce each other. Plate IX, Fig. 2, will make this plain. Here we have laid down in C on a black background a series of antipoints such as would be furnished by a file of radiant points seen through a circular aperture. If we now cut down the illumination of the image upon our retina, as we may either by looking at the figure through almost closed eyelids, or by looking at it through a piece of tissue paper, we see, instead of the discs and rings, a broad principal line and two flanking lines similar to those in Plate IX, Fig. 1, C.

Experiment. Raise a fine platinum wire to incandescence in the flame of a Bunsen burner, or as an alternative dispose a very fine bright needle upon the table in bright sunlight or lamplight in such a manner as to reflect light to the eye.

View the luminous line, which is in each case obtained, through a pinhole in a card and again through a narrow slit aperture disposed parallel to the object line.

Note that instead of the narrow luminous line which is obtained in the image in the case where we employ the full aperture of the pupil, we now obtain a broad line corresponding to the central discs of the file

Plate IX.

Fig. 1.

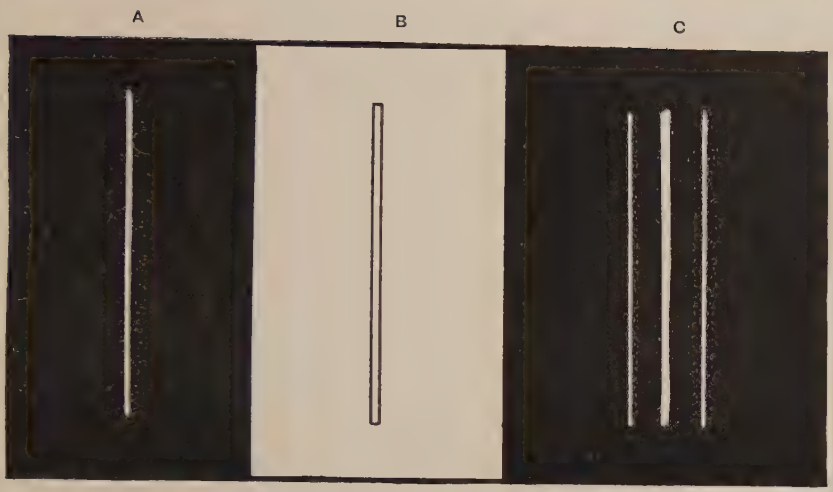


Fig. 2.

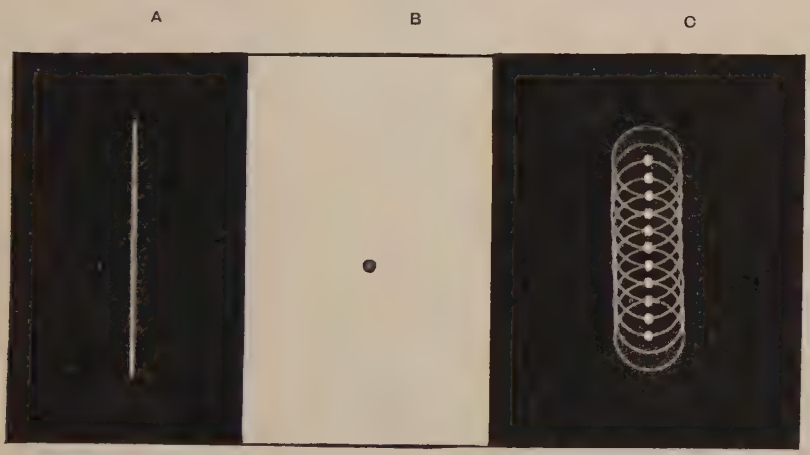
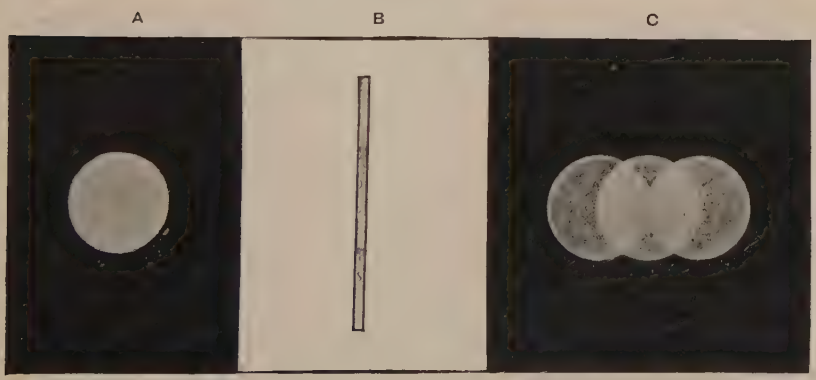


Fig. 3.



of antipoints, and two narrower and less luminous flanking lines corresponding to luminous elements derived from the first diffraction ring.

7. Antipoint pattern of a luminous surface.

Consideration will show that the antipoint pattern furnishing a luminous surface will correspond to that which would be obtained by apposing the antipoints of the constituent lines into which the surface in question can be resolved. Thus, the three discs shown in Plate IX, Fig. 3, C, would be arrived at by placing side by side the antipoints of the series of lines into which the disc in Fig. 3, A, can be resolved.

8. Dependence of the antipoint pattern, which is in each case brought into view, upon the brilliancy of the source of light, and distinction between "theoretical" and "conspicuous" antipoint.

In carrying out the experiments which have been described above, many indications will have been obtained of the dependence of the antipoint upon the luminosity of the object. In particular it may have been observed that the passage of a cloud over the sun conditions, in the case of the experiment with the mercury globule (*subsect.* 4 *supra*), the fading out of the ring system and the false disc, and in the case of the experiment with the needle (*subsect.* 6 *supra*) the fading out of the flanking lines and the attenuation of the principal line.

We have in point of fact, under conditions of ordinary illumination, to deal in the image not with the full antipoint which has been in question above, but with a mutilated antipoint, consisting of a false disc of reduced dimensions, shorn of its system of outlying rings. We shall find it convenient to speak of the antipoint which happens in the particular case to be conspicuous in the image as the "*conspicuous*," as distinguished from the "*theoretical*" antipoint.

The question will be further adverted to in *Appendix* I to this chapter, and again in *Caps.* X and XVI.

9. Law which governs the dimensions of the "theoretical" as distinguished from the "conspicuous" antipoint.

From the consideration of the pattern and the brilliancy of the antipoint pattern we pass naturally to the consideration of the dimensions of the antipoint, signifying—be it noted—here by the dimensions of the antipoint, the dimensions of any constituent in the antipoint pattern which may be agreed upon. It will be convenient in the following to understand by the dimensions of the antipoint, unless otherwise specified, the radius of the false disc,

i.e. the linear distance between the point of impact of the dioptric beam and the point of impact of the first caliginiferous beam.

The conditions which determine these dimensions will readily be understood on reference to the diagram below.

Let A B C represent the dioptric closing limb of a vista passing through the lens-armed aperture A B.[1]

Let E F, the radius of the circle described round the central point E of the aperture, correspond to half a wave-length of the particular light with which we are here dealing.

Let B F represent a tangent line falling upon the circle from the point B.

Let C D have been drawn parallel to A B.

Let E F D be a line drawn from the centre of the circle E through the point where the tangent line B F touches the circle and prolonged to meet the line C D.

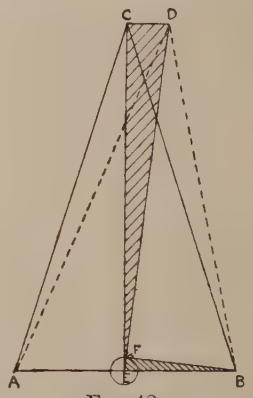


FIG 42.

The point D will be the point of impact of the first caliginiferous beam, and the distance C D will represent the radius of the central disc of the antipoint.

Proof. In the triangles E C D (distinguished in the diagram by rulings sloping obliquely upwards and to the right), B F E (distinguished in the diagram by rulings sloping obliquely downwards and to the right), the angles E C D, B F E are right angles, and the angles C E D, F B E are equal each to each, inasmuch as they are in each case completed to a right angle by the angle F E B.

$$\because \text{C E D} = \text{F B E} \therefore \text{sine C E D} = \text{sine F B E}.$$

$$\text{Sine C E D} = \frac{\text{C D}}{\text{E D}} \text{ and sine F B E} = \frac{\text{E F}}{\text{E B}}$$

$$\frac{\text{C D}}{\text{E D}} = \frac{\text{E F}}{\text{E B}}$$

$$\text{C D} = \frac{\text{E F} \times \text{E D}}{\text{E B}}$$

Dividing the fraction on the right above and below by E D,

$$\text{C D} = \frac{\text{E F}}{\text{E B} \div \text{E D}}$$

[1] While A B C may be taken to represent the median section of a conical beam, it is here discussed as if it were a wedge-shaped beam transmitted through a slit aperture. In point of fact a circular aperture would give an antipoint whose dimensions would be about one-fifth larger.

Substituting now

for C D, radius of central disc of antipoint;

for E F, half a wave-length, and calling this $\frac{1}{2}\lambda$;

for $\frac{E B}{E D}$, semi-aperture ÷ aperture-image distance—the numerical aperture of the closing limb—and calling this *n.a.*;

we arrive at the formula—

$$\text{Radius of false disc} = \frac{\frac{1}{2}\lambda}{n.a.}$$

By a similar line of argument we arrive at the formula—

$$\text{Radius of first bright ring} = \frac{\frac{1{\cdot}5}{2}\lambda}{n.a.} = \frac{{\cdot}75\,\lambda}{n.a.}$$

It will be noted that the longer the aperture-image distance, and the narrower the aperture, in short, the smaller the *n.a.*, the larger will be the dimensions of the antipoint.

Example. Required approximately the radius of the theoretical false disc and of the first bright ring in the case where a point which emits light of a wave-length of 0·0006 mm. is imaged through a circular aperture, 3 mm. in diameter, upon a receiving screen, set up 250 mm. behind the aperture.

$\frac{1}{2}\lambda$ is here 0·0003. ·75 λ is here 0·00045. *n.a.* is here $\left(\frac{1{\cdot}5}{250}\right)$ 0·006.

$$\text{Radius of false disc} = \frac{0{\cdot}0003}{0{\cdot}006} = 0{\cdot}05 \text{ mm. (approximately).}$$

$$\text{Radius of 1st bright ring} = \frac{0{\cdot}00045}{{\cdot}006} = 0{\cdot}075 \text{ mm. (approximately).}$$

10. Practical importance of the antipoint in connexion with the microscopic image.

The development of the antipoint may affect the microscopic image in three different ways :—

(1) Resolution is abolished where the central element (false disc) of one antipoint overlaps the central element (false disc) of an adjoining antipoint, and reinforces it in such a manner as to furnish uniform illumination over the region which corresponds to the overlap.

(2) *Spurious detail* is introduced into the image in the case where an outlying element of an antipoint is superimposed upon an outlying element of another antipoint, in such a manner as to reinforce it and bring it into prominence.

(3) Clear delineation, and, in particular, contrast between light and shade, is lost in the case where the antipoints, which

correspond to beams which traverse the aperture obliquely, spread inwards over the field in such a manner as to flood the image with diffuse light.

The first two of these points may be separately elucidated and illustrated by experiments. The latter will be referred to in connection with the adjustments required for the achievement of a critical image (*infra, Cap.* XV, *subsec.* 2).

11. On the central elements of the antipoint and on the loss of resolution entailed by the overlapping of the false discs of adjoining antipoints.

The experiments described below make clear the rôle played by the false disc in connexion with the resolution of the image.

It may be noted with regard to the experiments conducted with the microscope, that they involve the use of the Abbe diffraction outfit, as supplied by Zeiss, or of an extemporized outfit, as explained below. The outfit first referred to consists of (*a*) three systems of micrometric rulings on silvered discs, (*b*) appropriate diaphragms, and (*c*) a suitable adapter for mounting and rotating these diaphragms at the back of the objective. (Fig. 44.)

Experiment 1. Take a visiting card and—using a penknife or a pair of scissors—incise it in such a manner as to give a slit aperture when the edges of the cut are drawn apart. Placing before you now in bright sun- or lamp-light Fig. 43, which represents—in a magnified and simplified form—the central ruling of the Abbe diffraction plate, view the lines through this narrow slit aperture held vertically before the eye. An almost unresolved retinal image corresponding to Fig. 44 will now be obtained.

Carry round now the slit aperture until it lies transversely to the rulings. A perfectly resolved image corresponding to Fig. 43 will now be obtained.

Elucidation. The widening out of the bright lines into broad luminous bands when the slit aperture is disposed parallel to the rulings is a diffraction phenomenon exactly similar to that which has already come under notice in *subsect.* 6 (*supra*), where a single bright line was viewed through a slit aperture held parallel to its course.

It is to be noted that the diffraction element is present in the image whatever be the disposition of the slit aperture. If the antipoint is conspicuous only in the case where the slit is disposed parallel to the ruling, the reason of this is to be found in the circumstance that the thickening of the lines, which occurs in connection with this disposition of the slit aperture, involves encroachment upon the dark interspaces; while the lengthening out of the lines, which occurs when the slit aperture is disposed transversely to the direction of the rulings, involves no such encroachment.

Experiment 1a. (To be undertaken with the microscope where the Abbe diffraction apparatus is available.)

Place on the stage of the microscope the Abbe ruling; fit the adapter to the microscope tube and focus down upon the ruling with a one-inch objective giving a magnification in the diaphragm of the eye-

piece of not more than five diameters. Dispose the lines of the ruling in the antero-posterior meridian, and arrange them so that both closely and widely ruled lines lie in the field of view.

FIG. 43.

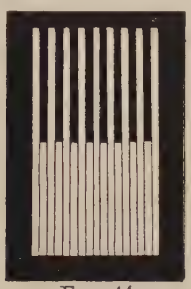
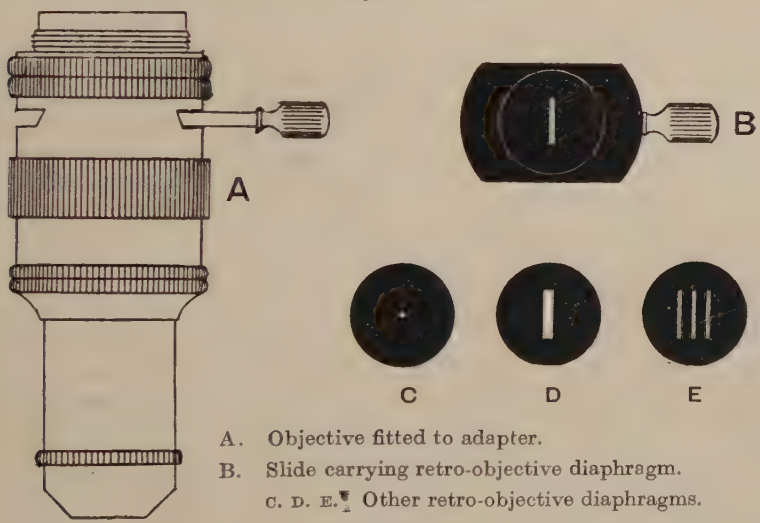


FIG. 44.

Now introduce into the slide (Fig. 45, B), which is designed to carry the diaphragm, the 1 mm. slit aperture, and, removing the eye-piece for the purpose of making the adjustment, dispose the slit aperture transversely to the direction of the rulings. Replace the eye-piece, and take note of the fact that the resolution of the picture is unaffected by the introduction of the stop. Now rotate the slit aperture through an angle of 90°. The ruling will now appear as in Fig. 44.

Having turned back the slit aperture into its original position, repeat the manœuvre, attending carefully to what happens. It will be observed that as the slit aperture is gradually carried round into parallelism with the rulings the fine lines of the original image are gradually widened. As they expand into broad bands the bright elements encroach upon the dark interspaces until, in the lower portion of the field, they entirely blot out the interspaces, and give us the unresolved image of the fine grating which is indicated in Fig. 44.



A. Objective fitted to adapter.
B. Slide carrying retro-objective diaphragm.
C. D. E. Other retro-objective diaphragms.

FIG. 45.

A simple calculation will show that the overlap of the central elements of the antipoint furnished by the slit aperture is, given brilliant illumination, more than sufficient to account for the obliteration of the fine rulings.

(*a*) Since we are dealing in the fine ruling of the Abbe grating, with lines positioned *circ.* 0·0073 mm. apart, we are therefore dealing in the 5-fold magnified image of this grating, with lines disposed *circ.* 0·038 mm. apart.

(*b*) Again, we are dealing with an image formed at a distance of about 120 mm., through a slit aperture 1 mm. in diameter.

Applying these data, we can calculate the dimensions of the antipoint in accordance with the formula (*subsect.* 9, *supra*)

$$\text{Radius of Antipoint} = \frac{\frac{1}{2}\lambda}{n.a.}$$

Substituting here for λ, 0·0006 mm., it is clear that here the radius of central element of the theoretical antipoint $\frac{0{\cdot}0003}{0{\cdot}5 \div 120} = 0{\cdot}072$.

(*c*) Bringing the elongation of the principal lines, as calculated in (*a*), into relation with the dimensions of the antipoint as calculated in (*b*), it will be manifest that the dark interspaces in the grating will be completely obliterated even when the illumination is much reduced.

Experiment 1B. (To be substituted for *Experiment* 1A, where the diffraction apparatus of Abbe is not available.)

Place the grating which is furnished with this book, and which is ruled with 16 lines to the millimetre, upon the stage of the microscope. Removing the eye-piece and fitting to the tube a one-inch objective, rack down the microscope tube until the rulings just come into view in accurate focus.

Arrange the rulings in the antero-posterior meridian.

Take a pair of parallel rulers and, opening them out so as to form a chink about 0·2 millimetre in breadth, place them with the slit aperture thus formed transversely across the open upper end of the microscope tube. The image of the rulings which is obtained on looking through this slit will not be less well resolved than the image obtained in the case where the rulings were viewed through the unrestricted pupil.

Now turn the parallel rulers through an angle of 90 degrees, so as to bring the chink into parallelism with the rulings. Resolution will be abolished.

Turn back the slit again in such a manner as to bring it obliquely across the rulings. Note that at a particular angle of turning resolution will be regained. The transverse diameter of the aperture at this point will represent the minimum breadth of aperture which will confine the visible false discs within the limits of size required for the resolution of the image furnished by the objective.

(*a*) Assuming that the transverse diameter of the slit aperture is 0·2 mm., the *n.a.* of the closing limb of the eye vista is here $\frac{0{\cdot}1\,\text{mm.}}{250\,\text{mm.}} = 0{\cdot}0004$.

The radius of the theoretical false disc is by the formula

$$\text{Radius of Antipoint} = \frac{\frac{1}{2}\lambda}{n.a.} = \frac{0{\cdot}0003}{0{\cdot}0004} = 0{\cdot}75\,\text{mm.}$$

(*b*) The separation of the bright lines in the object is 0·06 mm.; in the 10-fold magnified image it is 0·6 mm.

(*c*) The theoretical false discs will thus overlap the dark interspaces.

Experiment 2A. (To be undertaken with the microscope where the diffraction apparatus of Abbe is available.)

Retaining the slit aperture in the position of parallelism to the rulings in which it was left at the end of *Experiment* 1A, substitute for the objective employed in the last experiment an 8 mm. ($\frac{1}{3}$ inch) objective

giving in the diaphragm of the ocular an image magnified, say 15–20 diameters. Note that the fine lines can now be resolved.

The explanation of this fact is to be found in the circumstance that the centres of the false discs have now been carried apart to such a distance as to prevent, unless under quite exceptionally brilliant illumination, any overlap.

Utilizing the values obtained in the small-print paragraph appended to *Experiment* 1A, the dimensions which we have here to consider are as follows :—

Radius of the theoretical false disc, 0·072 mm.

Separation of the lines of the fine ruling in the object 0·0073 mm.

Separation of the lines of the fine ruling in the 15-fold magnified image 0·11–0·15 mm.

Experiment 2B. (To be substituted for *Experiment* 2A, where the Abbe diffraction apparatus is not available.)

Employing the extemporized apparatus used in *Experiment* 1B, and replacing the 0·2 mm. slit aperture above the ocular in a position of parallelism to the rulings, fit to the microscope an objective which gives, when employed directly with the eye, a picture magnified thirty times. An 8 mm. ($\frac{1}{3}$ inch) objective would do this.

Note that the rulings are now perfectly resolved.

Utilizing the values obtained in the small print paragraph appended to *Experiment* 1B, the dimensions we have here to consider are as follows :—

Radius of the false disc, 0·75 mm.

Separation of the bright lines in the object, 0·06 mm.

Separation of the bright lines in the 30-fold magnified image, 1·8 mm.

12. On the outlying elements of the antipoint considered in relation to the development of "spurious detail" in the image.

We may apply the term *spurious detail* to outlying elements of the individual antipoint, or, as the case may be, of the composite antipoint figure, which may impose upon the observer as substantive elements in the picture.

Spurious detail may, in the case where isolated points and lines are in question, take the form of circles, outlying dots and flanking lines, such as were under observation in the experiments in *subsecs.* 4, 5, and 6, *supra.* In the case where we are dealing with a system of parallel rulings, it may take the form of intercalated lines. In the case where we are dealing with a circle or subcircular figure, it may take the form of spots inserted centrally into the figure.

Inserted lines and spots, such as are here in question, are, in the technical language of microscopy, spoken of as *intercostal lines and spots.*

The latter may here be considered. The former will be more appropriately considered in connexion with the diffraction patterns produced by gratings.

13. Development of intercostal spots.

An intercostal spot is developed when a file of luminous points, disposed in the form of a circle, is viewed through a circular aperture under conditions where an antipoint is obtained, which conforms, with respect to the dimensions of its primary ring, with the dimensions of the dioptric image of the circle. This is made plain in Plate VIII, Fig. 3, C, which represents the composite antipoint pattern which would be produced under these circumstances. Of this pattern only the brighter elements, which are seen when the figure is viewed through the almost closed eyelids or through a piece of tissue paper, are actually brought into view. These brighter elements consist, as will be seen, of a broad circular band of light—corresponding to the false discs—and of a central spot corresponding to the point of overlap of the primary diffraction rings.

Experiment. When an electric lamp is available, take up a position such that the glowing filament comes into view in the form of a luminous circle; or, in default of an electric lamp, form a circle at the end of a piece of platinum wire and raise it to incandescence in a Bunsen burner. Place now a very minute pin-hole opening before the pupil. Note that the filament appears thickened. This is in conformity with the fact that the luminous points are now represented in the retinal image by comparatively large false discs. Now recede farther and farther from the lamp so as to cause the dimensions of the dioptric image on the retina to shrink while the dimensions of the antipoint remain unaltered. When by these means the dimensions of the dioptric image of the circle and of the first ring of the antipoint exactly correspond, a luminous *intercostal* point will appear in the centre of the figure.

14. Antipoint patterns furnished by diffraction gratings.

As above indicated, we may most conveniently study the development of intercostal lines by the aid of diffraction gratings. *For the purposes of the necessary experiments, a simple form of grating*[1] *is provided in the pocket of the cover of this book.*

As compared with a simple restricted aperture, a diffraction grating has for the purposes of our contemplated experiments two advantages: First, it furnishes conspicuous antipoint patterns, with objects of comparatively low luminosity. Secondly, in the case of the antipoint patterns, obtained by a diffraction grating, the outlying elements of the antipoint, i.e. the elements of the antipoint which we are here for the moment interested in, stand out much more conspicuously than they do in the case of the antipoint figures developed with simple restricted apertures.

[1] A more permanent form of this diffraction grating can be obtained from Messrs. Sanger Shepherd, Gray's Inn Place, London, W.C.

15. Preliminary experiments with the diffraction grating.

Experiment 1. Place the diffraction grating with its lines disposed vertically immediately in front of the eye and view through this a distant candle flame. Note that in lieu of the one or two parts of spectral images which are obtained by a simple slit aperture a whole series of such repetitions is obtained.

Experiment 2. Place again the grating with its rulings disposed vertically immediately in front of the pupil and view through it a white dot or line inscribed upon a black background, or alternatively, and more conveniently, a dark dot or line inscribed on a white background. Note—and this exemplifies one of the advantages of the grating as compared with the simple restricted aperture—we obtain here a manifest antipoint. Even in subdued light one pair of flanking dots or, as the case may be, one pair of flanking lines (Fig. 46 *infra*) will be very clearly visible.

Experiment 3. Place again the diffraction grating with its rulings vertical in front of the eye and view through it a dot placed

FIG. 46.

Object. Enlarged representation of diffraction grating. Antipoint pattern as obtained in the image.

on paper. Now rotate the grating and note that, as was the case with the simple linear aperture, the flanking points rotate (maintaining always their position on the normal to the rulings) in such a manner that when the grating has been rotated through two right angles there will have been described by the flanking points a ring system similar to but brighter than that which would have been obtained with a simple circular aperture.

Experiment 4. View through the grating with its rulings disposed vertically as in Fig. 46, a long line also disposed vertically. Having taken cognizance of the elongation of the flanking line, rotate the grating into the position shown in Fig. 47, and note that the flanking lines come in closer to the principle line and are arranged as in Fig. 47, c.

A comparison of Figs. 46, and 47, shows that the reduction in the elongation of the flanking lines of the antipoint pattern which is associated with the rotation of the grating is dependent upon a widening out of the centre-to-centre distance between its apertures along the horizontal line.

A comparison of the figures further brings out the fact that the elongation of the outlying element A′ on the flanking line from central element A on the principal line measured in each case upon the normal to the rulings of the diffraction grating (A′ A′) is in each case exactly the same.

We have accordingly here to deal, not with a reduction in the dimensions of the individual antipoint but, with the different disposition of its component elements.

Experiment 5. Viewing the same object through the grating with its rulings disposed vertically, tilt forward the right or left edge of the grating till its ruled surface forms an acute angle with the axis of vision. It will now be found that the elongation of the flanking lines has increased. When the grating is tilted in this manner a larger number of apertures are brought in front of the eye, each of these being foreshortened, with the result that the dimensions of the antipoint are increased in the same manner as if a finer had been substituted for a coarser grating.

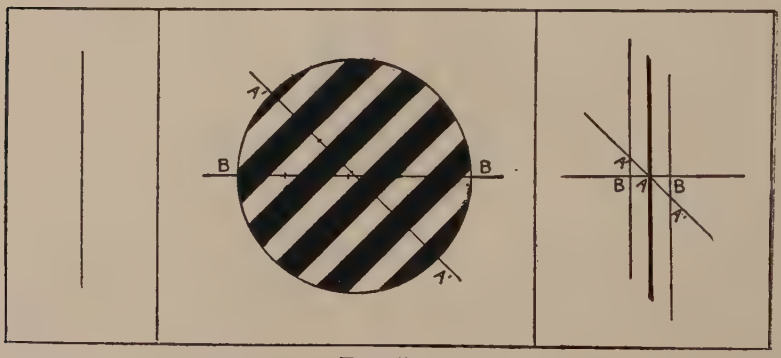


FIG. 47.

Object. Enlarged representation of diffraction grating. Antipoint pattern as obtained in the image.

16. Dimensions of the antipoint pattern furnished by a grating. The only measurement with which we shall have to concern ourselves in connexion with the antipoint of a diffraction grating is the elongation of the flanking point or, as the case may be, flanking line. The elongation of this point on the retina, or other receiving screen, is furnished, not by the formula which applies to the case of an uninterrupted wave-front and a simple aperture (Fig. 41, E–H and Fig. 42), but by the formula which applies where we are dealing with isolated rays (Fig. 41, A–D). The applicability of the formula last mentioned to the case of the diffraction grating will be recognized when it is appreciated that each of the narrow

apertures of a diffraction grating transmits what is in effect an isolated ray. It follows that the elongation of the flanking point or line can in the case of a diffraction grating be obtained by the formula which would be applicable to Fig. 41, D.

$$\text{Elongation of first flanking point or line} = \frac{0{\cdot}5\ \lambda}{n.a.}$$

$n.a.$ standing here for $\frac{\frac{1}{2}\text{ centre-to-centre distance of the ruling}}{\text{aperture-image distance.}}$

17. Examples and verification of this formula for the dimensions of the antipoint of a grating.

Example 1. Required the elongation of the flanking lines in the grating of the antipoint furnished by a diffraction grating where the centre-to-centre distance (i.e. the distance measured from the centre of one aperture to the centre of the next) between the lines is 0·0625 mm. and where the image is projected upon a screen set up at a distance of 1 metre from the diffraction screen.

Taking $\frac{1}{2}\lambda = 0{\cdot}0003$ mm.; $n.a.$ as $\left(\frac{0{\cdot}0312}{1000}\right) = 0{\cdot}0000312$, and dividing the former of these by the latter, we obtain the answer.

Elongation of the flanking line = 9·6 mm. nearly.

Verification. Place before you Fig. 48. After verifying that the outermost fiduciary line to the right is disposed at a distance of 9·6 mm. from the vertical line, set up the figure in a bright light at a distance of 1 metre from the eye, and view it through the grating which is supplied in the cover of this book. The apertures upon this grating are disposed ·0312 mm. apart, giving a centre-to-centre distance of 0·0625 mm. ($\frac{1}{400}$ inch).

Fig. 48.

Dispose the grating so that the horizontal line may be seen without flanking lines and in perfectly sharp definition. In this position of the grating the flanking lines of the upright line will have attained their maximum elongation, and the flanking line on the right will be found to fall in the longitude of the outer fiduciary line, which is, as we have seen, disposed at a distance of 9·6 mm. from the upright line.

Example 2. Required the elongation of the flanking line in the antipoint furnished by the same grating in the case where the image is formed at a distance of 250 mm. from the aperture of the grating.

In conformity with the fact that the n.a. is in this case four times greater than it was in the last example.

Elongation of the flanking line = 9·6 ÷ 4 = 2·4.

Verification. Employing again Fig. 48 and verifying that the inner fiduciary line to the right is disposed at a distance of 2·4 mm. from the

vertical line, take in hand a rule divided into millimetres, and place it on end upon the figure. Now place the diffraction grating in position at 250 mm. from the eye and look through it after bringing its rulings into parallelism with the vertical line. The flanking line will now coincide in position with the fiduciary line which has been in question above.

Example 3. Required the elongation of the flanking lines in the case where the grating has a centre-to-centre distance between the apertures of 1.6 mm. and where the image is formed 120 mm. behind the grating.

In the formula $\frac{0{\cdot}5\,\lambda}{n.a.}$, $0{\cdot}5\,\lambda$ is here, as before, 0·0003 mm.; and $n.a.$ is $\left(\frac{0{\cdot}8}{120}\right) = 0{\cdot}0066$. Dividing the former by the latter expression we obtain as the answer—

Elongation of the flanking line 0·045 mm.

Verification. The verification of this result is furnished in connexion with *Experiment* 5, *subsect.* 18 *infra.*

18 Development of intercostal lines in such a form as to produce doubling or trebling in a periodical ruling.

Where the conditions are such that the individual line is represented in the image by a conspicuous antipoint, we have conditions which may in the case of a periodical ruling result in its doubling or trebling by the insertion of intercalated lines.

The ruling will be doubled in the case when in the image the elongation of the flanking line from the principal line corresponds to half the periodic interval of the principal lines.

The appropriate adjustment of these two dimensions may in any particular case be made by (*a*) increasing or, as the case may be, diminishing the separation of the principal lines in the image, and (*b*) by diminishing or, as the case may be, increasing the elongation of the flanking lines. The first method of adjustment involves a regulation of the scale of the dioptric image; the second a regulation of the dimensions of the antipoint.

The following experiments will bring clearly before the understanding the conditions which bring about in the case of a periodical ruling the coalescence and mutual reinforcement of the flanking lines of different antipoint systems.

Experiment 1. View through the diffraction grating held with its rulings vertical, two vertical lines positioned 9·6 mm. apart on a sheet of paper. Dispose the figure at a distance of 250 mm. from the eye.

Two separate antipoint figures, consisting in each case of three lines separated by a periodic interval of 2·4 mm., will come into view.

Now set back the sheet of paper until it is 500 mm. from the eye. The two inner flanking lines, which have been gradually approaching each other as the paper was being carried away from the eye, will now overlie each other and will by their coalescence form a line comparable in distinctness to the principal line.

Set back now the sheet of paper to one metre from the eye. The inner flanking of each antipoint will now be superposed upon, and will merge with, the principal line of the companion antipoint, giving a composite antipoint system consisting of only four lines.

Elucidation. When the object is set back from the eye, the scale of the dioptric image on the retina is progressively reduced, while in each case the dimensions of the antipoint are maintained.

The progressive reduction in the scale of the image is in conformity with the fact that the length of the opening limb of the vista (distance of object from the pupil) is here first doubled and then quadrupled, while the closing limb of the vista (distance from diffraction grating to retina) remains constant. The size of the antipoint—here the elongation of the flanking lines in each case from their principal lines—remains constant, in accordance with the fact that the numerical aperture of the closing limb of the vista,

$$\frac{\frac{1}{2}\text{ periodical interval of the diffraction grating,}}{\text{Length of closing limb of vista}}$$

is unaltered.

Experiment 2. Maintaining the object lines at a distance of one metre from the eye, rotate the grating until it forms an angle of 60° with the vertical. The periodical interval of the ruling will now be halved.

Elucidation. The alteration in the retinal image which is effected by the rotation of the grating is due to a reduction in the elongation of the flanking lines, not to any alteration in the separation of the principal lines in the retinal image. The fact that the elongation of the flanking images is diminished by half stands, in relation to the fact that the rotation of the grating through an angle of 60° exactly doubles the horizontal centre-to-centre distance between the apertures of the grating.

Experiment 3. Placing before you Fig. 49, which again represents the central ruling of the Abbe diffraction plate, view it from a distance of about 400 mm. through the diffraction grating disposed with its rulings vertical. After a little adjustment of the distance, the rulings both in the upper and lower half of the field will be seen uncomplicated by spectral lines, but supplemented on either side of the figure by an additional ruling in the upper half of the field and an additional pair of rulings in the lower half of the field. Rotate the grating through an angle of 60° so as to double the centre-to-centre distance between its apertures. The short lines of the

Fig. 49.

fine ruling are now prolonged into the upper half of the field in such manner as to bisect the bright interspaces between the more widely interspaced rulings, as shown in Fig. 50. On each side of the figure there will be now, both in the upper and lower halves of the field, a single additional ruling.

Elucidation. The explanation of the fact that the picture is in the first position of the figure and grating, seen uncomplicated by intercostal lines, is to be attributed to the fact that, owing to the circumstance that the elongation of the flanking lines here corresponds with the periodical interval of the coarser ruling, as depicted in the retinal image, the flanking lines of the more widely interspaced rulings are in each case superposed upon the principal lines next in series on either side; while they are in the case of the fine rulings superposed upon the principal lines next but one in series on either side.

When the grating is rotated through an angle of 60°, the figure remaining at 500 mm. from the eye, the elongation of the flanking lines in the retinal image corresponds in each case to the periodical interval of the fine ruling.

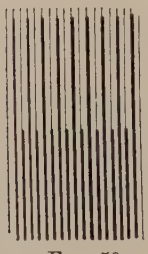


Fig. 50.

Fig. 51.

It follows from this that the flanking lines of the fine ruling fall in each case upon the principal lines next in series, while the flanking lines of the coarse grating in each case coalesce in such a manner as to form an intercostal line continuous with the short line of the finer ruling.

Experiment 4. Placing before you again Fig. 49, view it from a distance of about 200 mm. through the diffraction grating disposed with its rulings vertical. Note that the image obtained now corresponds to that shown in Fig. 50, except only in the respect that the lines here end all upon one level instead of slanting off as they do in that figure.

Again rotate the grating through an angle of 60°. The rulings are now, as shown in Fig. 51, doubled in the lower half, and triplicated in the upper half of the field.

Elucidation. The explanation of the fact that the image obtained with the figure disposed at 250 mm. from the eye, and the grating held with its rulings vertical, corresponds (except in the points of detail mentioned above) with the image obtained (in *Experiment* 3) with the figure disposed at a distance of 500 mm. from the eye, and the grating rotated through an angle of 60°, is to be found in the fact that here we have in conjunction with the image which corresponds to an object placed at the near point of vision the antipoint which corresponds to a diffraction grating of 16 lines to the milli-

metre; whereas in *Experiment* 3, we have in connexion with a two-fold reduced image the two-fold reduced antipoint which corresponds to a grating of 8 lines to the millimetre.

Experiment 5. Place the central ruling of the Abbe diffraction plate on the stage of the microscope under a 16-mm. objective. Arrange it so that the lines are disposed in the antero-posterior meridian and that the fine rulings shall occupy the lower, the coarser ruling the upper half of the field.

Now introduce behind the objective—employing for this purpose the diaphragm carrier of the Zeiss diffraction apparatus—the diaphragm with three slit openings [1] (Fig. 45, E.) which is provided with the Abbe apparatus.

In the upper half of the field each line will now be imaged, as in Fig. 51, by three lines.

In the lower half of the field, i.e. in the half of the field occupied by the fine lines, the ruling will be doubled.

Elucidation. We are here employing a diffraction grating with a centre-to-centre distance of 1·6 mm.

The figure obtained in the upper half of the field is, of course, made up of a series of principal lines, furnished on either side with flanking lines.

The figure in the lower half of the field is the result of the superposition of the flanking lines of adjacent antipoints. It will be seen that the intercostal rulings of the lower ruling are in line with the flanking lines of the upper system of ruling. The appearances are in conformity with the data given below.

(*a*) Separation of the fine lines in the ruling upon the stage of the microscope —0·0073 mm.

Separation of the fine lines in the 12-fold[2] magnified image = 0·088.

(*b*) Elongation of the flanking lines, by the formula $\frac{\frac{1}{2}\lambda}{n.a.}$, where the centre-to-centre distance between the apertures in the grating is 1·6 mm., and the aperture-image distance is 120 mm. $\frac{0{\cdot}0003}{0{\cdot}8 \div 120} = \frac{0{\cdot}0003}{0{\cdot}0066} = 0{\cdot}045$ mm.

[1] The fact that the diaphragm with three slit openings which is here in question functions as a diffraction grating was pointed out by Mr. Gordon (*Proc. Royal Microscopical Soc.* 1901).

[2] This figure is approximate only.

Appendix I.

ON ABBE'S THEORY OF MICROSCOPIC VISION AND THE EFFECT OF DIFFRACTION OCCURRING IN THE OBJECT PLANE AND OPENING LIMB OF THE VISTA.

The diffraction which occurs in the aperture of the lens and closing limb of the vista has been treated of in the preceding chapter. We have seen that it constitutes an obstacle to resolution.

In the theory of microscopic vision, which is associated with Professor Abbe's name, diffracted light plays a very different rôle —the rôle of an adjuvant to resolution.

In the earlier form of Abbe's theory all detail in the microscopic image was declared to be revealed by diffracted, as distinguished from dioptric, light. In the later version of the theory—and this is the only version of the theory we have here to consider—it is contended that the object "as it really is," is revealed only when a "full complement" [1] of diffracted rays is gathered in to the image.

This contention, which is equivalent to a contention that spurious resolution can be avoided and point-for-point representation can be approximated to only in the case where diffracted light contributes to the image, appears at first sight to be in flagrant and hopeless conflict with the facts of which we have taken cognizance in the preceding chapter. This is, however, not so. Careful examination proves that there is here confusion—not contradiction.

In reality, Professor Abbe's contention has in view not the diffracted light which comes off from the aperture and closing limb of the objective vista, but the diffracted light which comes off from the stage of the microscope and the opening limb of the objective vista.

The fact that two entirely distinct systems of diffracted rays are here in question, may be brought clearly before the eye by the aid of Fig. 52.

We have brought to focus here, by the condenser upon the

[1] For the signification which is attached by Abbe to this term, *vide infra* Footnote to *Experiment* 1.

microscope stage, a very narrow beam. We may speak of this as the dioptric beam. It takes, as will be seen, a new departure from the focal point on the stage, and passes on to enter the objective.

Radiating out on every side from the focus of this dioptric beam, we have a system of diffracted rays. These diffracted rays, which are indicated in the diagram by broken lines, are those which Professor Abbe has in view when he declares that diffracted rays are essential to satisfactory resolution. They are shown in the diagram as passing into the aperture of the lens, forming, as it were, a casing round the beam we have spoken of as the dioptric beam. From the aperture of the lens a second and quite independent system of rays is seen to take origin. These are those which—furnishing as they do the antipoint considered in the preceding chapter—constitute as shown an impediment to resolution.

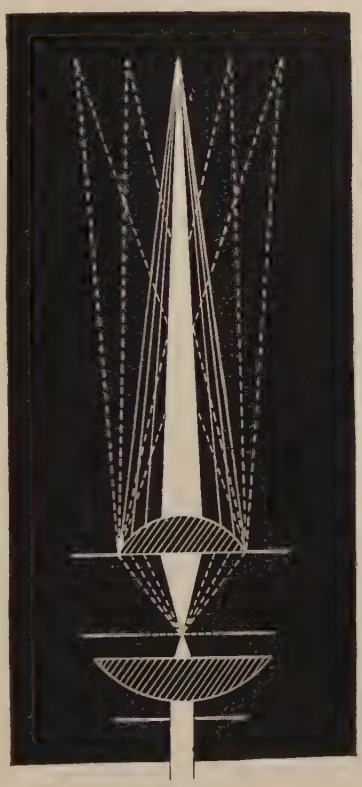


FIG. 52.

The study of the above diagram may be usefully supplemented by an experiment which brings the diffracted light, which Professor Abbe has in view, directly to the reader's cognizance.

Experiment 1. Place upon the stage of the microscope either an isolated bright ruling inscribed upon a black background, or preferably—for this will yield a better diffraction pattern—a series of fine rulings. Such a system of rulings is furnished by the rulings of an ordinary stage micrometer, or by rulings inscribed on the central disc of the Abbe diffraction plate. For the purposes of the present experiment we will assume that of the Abbe diffraction plate is employed, and that its rulings are arranged in the antero-posterior meridian. This done, focus the condenser upon the microscope stage, as explained in *Cap.* XIII, *subsect.* 5, and stop down the aperture of the substage to very narrow dimensions either by means of the iris diaphragm, or by means of the small circular or linear diaphragm supplied with the Abbe diffraction apparatus—remembering in the case where the linear diaphragm is employed to dispose this parallel to the rulings of the diffraction plate.

Now focus down upon the fine lines of the central ruling with a one-inch objective. This done, take a pocket lens before the eye, and, bending down over the microscope, examine by its aid the apertural plane of the eye lens. There will be imaged here, in accordance with the principle explained in *Cap.* VII, *subsect.* 37, the apertural plane of the objective vista, and in this the apertural plane of the condenser vista.

This last, into which there has been inserted, as described above, a circular or linear diaphragm, will come into view as a luminous disc or line flanked on either side by a number of spectral [1] lines or discs. These discs will, when they are large, overlap as in Plate VIII, Fig. 3. When they are small they may be spaced out as indicated in the inset in Fig. 53.

Keeping the back of the objective under observation, remove now the ruling from the stage of the microscope.

The flanking images will disappear while the principal or dioptric image of the stop will remain filling out only a small area of the aperture of the objective.

Elucidation. Two different images come into consideration in this experiment: first, the image (B′A′, Fig. 53) of the Abbe diffraction plate, which is seen on looking down the microscope in the ordinary way; secondly, the image (D″C″, D′C′, D″C″, Fig. 53 and inset) of the substage diaphragm, which is seen on viewing the Ramsden disc of the eye-lens through a magnifying lens.

The rulings on the stage of the microscope stand in an entirely different relation to the two images and the two catenae of vistas which come into consideration.

In relation to the principal catena and the image seen on looking down the microscope in the ordinary way, the rulings count as elements in the plane of origin of the catena and as constituent elements of the object picture.

In relation to the catena of the Ramsden discs and the image seen on viewing the Ramsden disc of the eye lens through a magnifying lens, the rulings count only as a diffraction grating positioned in the apertural plane of the vista which has its plane of origin in the substage diaphragm and its terminal plane in the aperture of the objective.

In correspondence with the above, the diffracted rays which take origin in the apertures of the grating fulfil in connexion with the two images which come into consideration in each case a different rôle.

In connexion with the image of the object on the stage, the diffracted rays come into consideration as rays, which reinforcing and adding breadth to the dioptric beam, come into focus in conjunction with it.

In relation to the image of the substage aperture the diffracted rays play a much more conspicuous rôle. They dissociate themselves from the dioptric beam and come to focus in the terminal plane of the condenser

[1] When three of these spectral images are developed on each side of the principal image, the dioptric beam is, in accordance with the Abbe theory, to be regarded as furnished with a full complement of diffracted rays.

vista in such a manner as to furnish on either side of the principal image of the substage aperture one or more spectral images.

The manner in which the diffracted light which comes off from the grating on the stage is built up into the spectral images of the diaphragm which are developed at the back of the objective is made clear in the diagram.

We have now cleared the ground, and are in a position to consider the dictum that adequate and non-fallacious resolution can be obtained only in the case where rays diffracted at a considerable angle from the optical axis contribute to the image.

This dictum will, as consideration will show, be rebutted if it can be shown :—

(*a*) that satisfactory resolution can be obtained from a lens, quite apart from the supplementation of the dioptric beam, by diffracted light taking origin upon the stage of the microscope.

(*b*) that an unresolved image may be obtained, even when the dioptric beam from the stage has been supplemented in the amplest manner by diffracted light.

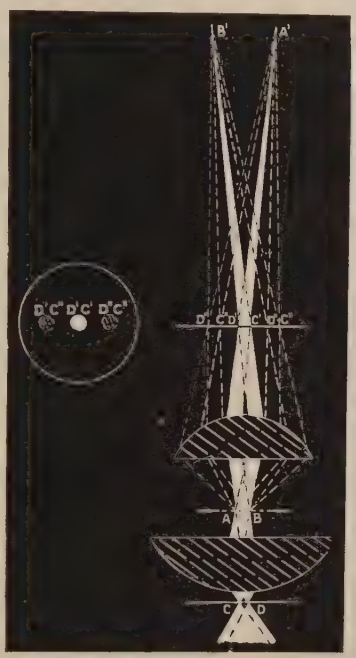


Fig. 53.

These propositions are established by the following experiments :

Experiment 2. Make the same dispositions as in *Experiment* 1, omitting only the diaphragm which was there employed in the substage.

Having satisfied yourself that a satisfactorily resolved image of the object upon the stage is obtained, bring into view with a pocket lens the back of the objective, and note that the spectral images of the stop no longer appear. Further note that the aperture of the objective is now fully filled in with the dioptric beam.

Verify the fact that the diffracted rays here fall entirely out of account by taking cognizance of the fact that when the diffraction grating is withdrawn from the stage no change is seen in the image of the substage in the apertural plane of the objective.

Experiment 3. Set up the microscope in direct sunlight, making in every other respect the same dispositions as in *Experiment* 1.

Look down the microscope and take note of the fact that the fine lines of the ruling are now unresolved.

Now bring into view as before the image of the substage diaphragm which is formed in the Ramsden disc of the eye lens, and take cognizance of the fact that we have here, as in *Experiment* 1, in addition to the principal image, a whole series of spectral images of the substage stop.

Elucidation. The formation of the spectral images of the stop shows that the dioptric beams which enter the lens are here furnished forth with a full complement of diffracted rays.

The circumstance that the image of the grating is here unresolved in spite of the amplification of the dioptric beam by diffracted rays is due to the circumstance that the antipoint of the image is here the larger antipoint which corresponds to the pre-eminently brilliant dioptric beam and not the smaller antipoint which would correspond to this beam amplified by the diffracted light from the stage of the microscope.

Conclusions with respect to the theory of Abbe, and with regard to the true import of the experiments by which that theory was supported.

It has been shown in the above that the dictum of Abbe that satisfactory and trustworthy resolution cannot be obtained apart from the participation of diffracted light in the formation of the image, is in conflict with the facts.

It remains only to consider the experimental procedure followed by Abbe and the import of the data which those procedures furnished.

The experimental procedure by which Abbe obtained the data which he has adduced in support of his theory was as follows :—

He narrowed down, as in *Experiment* 1 (and Fig. 52), *supra*, the aperture of his condenser in such a manner as to transmit to the microscope stage only a narrow beam. He then supplemented the narrow dioptric beam thus achieved by disposing his diffraction plate upon the stage of the microscope.

Having obtained in this manner a beam composed of a core of dioptric light, enveloped in a casing of diffracted light, he investigated the effect of shutting off, by means of a retro-objective stop, the outlying portion of the beam which filled out his objective. The deterioration and improvement of the image which were observed to follow the shutting off or admission of the peripheral portion of the beam were then attributed to the shutting out and admission of the diffracted component of the beam.

It was left to Mr. Gordon [1] to draw attention to the fact that absolutely identical effects are, as we have seen in connexion with *Experiment* 1*a*, *subsect.* 11, *supra*, obtained by the shutting off and

[1] Gordon, *Journal of the Royal Microscopical Society*, 1902.

transmission of the peripheral portion of the beam, in the case where this last consists exclusively of dioptric light.

It follows that the effects which were by Abbe attributed to elimination or, as the case may be, to the supplementation of the N.A. of the dioptric beam by diffracted light are in reality imputable to alterations in the dimensions of the antipoint standing in relation with the narrowing or widening of the *n.a.* of the objective vista. In other words, the changes in the image which are produced by the insertion of a retro-objective stop stand in relation to diffraction phenomena occurring in the aperture and closing limb of the objective vista; and not in relation to diffraction phenomena occurring in the plane of origin and opening limb of that vista.

Appendix II.

ON THE DEVELOPMENT OF THE MEDIAN INTERCOSTAL LINE CONSIDERED AS A FIDUCIARY PHENOMENON UPON WHICH ACCURATE ADJUSTMENT MAY BE MADE IN MAKING OPTICAL MEASUREMENTS.

(1) A narrow and sharply defined median intercostal line is, as has been shown above (*subsect.* 18), developed in a ruling when it is viewed through a diffraction grating, which furnishes an antipoint in which the elongation of the flanking lines from their principal lines corresponds to exactly half the interval between those principal lines.

(2) The median intercostal line here obtained will be resolved into its elements : (*a*) in the case where the scale of the dioptric image, and in correspondence with this the interspacing of the principal lines, is changed while the dimensions of the antipoint are maintained unaltered ; (*b*) in the case where the dimensions of the antipoint, and in correspondence with this the elongation of the flanking lines from their principals, is increased or diminished while the scale of the dioptric image remains unaltered.

(3) A median intercostal line will, in either case, be reconstituted on substituting for the system or pair of rulings originally under consideration a system or pair of rulings which is so interspaced as to give, under the conditions which have effected the breaking up of the original median intercostal line, an image in which the principal lines of the substituted rulings are spaced out to double the distance of the flanking lines.

(4) The quotient obtained by dividing the interval, or, as the

case may be, periodical interval of the substituted rulings into the interval or periodical interval of the discarded rulings, furnishes an exact measure of the alteration effected in the scale of the image, or, as the case may be, in the scale of the antipoint.

Experiment 1.[1] Hold the diffraction grating supplied close up to the eye with its rulings vertical, and view through it from a distance of *circ.* 200 mm. the sixth pair of lines in the figure below. These lines, spaced out as they are 4 mm. apart, will give, as seen through the grating, a sharply defined median intercostal line.

Fig. 54.

Maintaining the diffraction grating in position as before, take in hand a pocket lens and bring it in position in such a way as to give upon the retina a magnified image of the pair of lines under consideration.

It will be seen that the principal lines are carried apart, and that they carry with them, as they move outwards, in each case their flanking lines, with the result that the median intercostal line is resolved into its elements.

Maintaining the lens at the same working distance, search through the figure until a pair of lines is found which gives, as seen through the lens and diffraction grating, a sharply defined intercostal line.

Measure now the linear distance between the lines which furnish to the unaided eye a median intercostal line, and again the linear distance between the lines which furnish a similar intercostal to the lens-armed eye.

Dividing the first of these measurements by the second, the magnifying power of the lens, as employed in the experiment, is obtained.

Experiment 2. Hold the diffraction grating before the eye with its rulings forming an angle of 45° with the vertical, and search the figure above for a pair of lines which gives with the diffraction grating held at this angle a median intercostal line.

Taking now to aid a magnifying lens, spread out the principal lines so as to draw apart and leave a gap between the previously superposed flanking lines.

Now rotate the grating in such a manner as to bring its rulings up in the direction of the vertical. The diminution of the periodical interval of the grating, which will thus be effected, will increase the elongation of the flanking lines in such a manner as to span over at one stage of the rotation the interval between the principal lines and to reconstitute a median intercostal line.

[1] Wright, *Journal of the Royal Microscopical Society*, 1903.

The situation is now such that, if we know the periodical interval of the grating and can measure the angle of rotation, we can from this calculate the elongation of the flanking line in each case from its principal line. The multiplication of this elongation by two will give us the distance between the principal lines in the image.

Or, alternatively, if we can measure the interval which divides the principal lines in the image, we can, by dividing two, arrive at the elongation of the flanking lines, and calculate from it the centre-to-centre distance between the slit apertures, or, in the case where this is known, the angle through which that grating has been rotated.

Experiment 3. Holding the diffraction grating as in *Experiment* 1, view through it from a distance of 250 mm. the V on the right of the diagram. Note that the interior arms of the spectral Vs intersect in the fork of the dioptric V at the level of the third fiduciary mark where the transverse diameter of the V corresponds to the interval between the widest part of lines.

Maintaining the diffraction grating in place, now view the V through a pocket lens, placed as before at its full focal length from the figure, and note that owing to the spreading out of the limbs of the V the point of intersection now falls at a lower point in the fork of the V.

Removing the lens and withdrawing to a greater distance, view the V through the diffraction grating, and note that, owing to the diminished size of the dioptric image on the retina, the point of intersection of the spectral Vs now falls high up in the fork.

CHAPTER X.

IMAGE FORMATION IN THE CASE WHERE THE OBJECT IS VIEWED BY THE UNAIDED EYE.

Introductory—Inversion—Scale of the retinal image—Psychological factors involved in the estimation of the scale and disposition of the retinal image—Quality of the image furnished by the eye—Resolving power of the normal eye—Factors upon which the quality of the image depends—Numerical aperture of the eye—On the effect exerted upon the retinal image by a reduction of the numerical aperture of the eye—Significance of the results arrived at in the foregoing experiments in relation to the question of the limitation of resolution by the aperture of the objective—Question of the existence of an absolute physical limit to the resolving power of the eye, irrespectively of such limitations as may be imposed by the structure of the retina—Projection picture of the pupillary aperture.

1. Introductory.

While the photographic camera furnishes a focussed image, the microscope furnishes at the eye lens a system of pencils of parallel rays, which are brought to focus by the observer's eye.

Inasmuch as the eye is in this manner an integral optical element in the microscope, we may conveniently begin by a study of its optical system.

In the case of ordinary vision, we have, as the figure below makes clear, to deal with a single vista. This vista has its plane

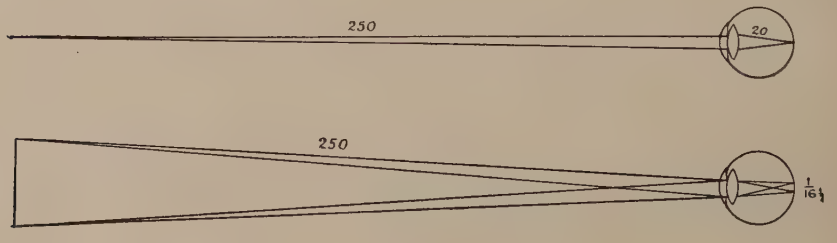


FIG. 55.

THE OBJECT IS HERE ASSUMED TO BE DISPOSED AT THE NEAR POINT OF VISION 250 MM. (10 INCHES) IN FRONT OF THE EYE.

of origin in the object ; its apertural plane in the pupil ; and its terminal plane upon the retina.

2. Inversion.

In consequence of the crossing of the beams which takes place in the aperture of the pupil, the image is, as shown in Fig. 55, laid down upon the retinal screen in the inverted position.

3. Scale of the retinal image.

Remembering that the scale of the image can, in the case of every vista, be obtained by dividing the numerical aperture of its opening limb by the numerical aperture of its closing limb, and remembering that the image in the case of the eye is formed in a medium which possesses the refractive index of water, 1·3, we can obtain the scale of the retinal image by dividing the numerical aperture of the opening limb of the eye vista, which may, as we shall see (*subsect.* 8 *infra*), be taken as 0·006, by the numerical aperture of the closing limb of the eye vista, which may, as we shall see, be taken as 0·1 nearly. The retinal image worked out in this manner is a $16\frac{1}{4}$-fold minified image.

4. Psychological factors involved in the estimation of the scale and disposition of the retinal image.

What has been said above with regard to the inversion and scale of the retinal image has reference to it considered as an objective optical phenomenon. There comes into consideration, in connexion with the estimation of the retinal image, also a psychological factor. We are accustomed to project the retinal image outwards to its source in the external world, and to treat this virtual image as if it were the true visual image.

Proceeding in this manner, we think and speak of the visual image as erect, when in point of fact the retinal image is inverted. In like manner, we speak of the visual image as inverted when the retinal image is erect.

Following out the same system, we think and speak of the visual image as equivalent in size to the object, when in point of fact the retinal image is reduced $16\frac{1}{4}$ or more times ; according as the object is situated at the near point of vision, or, as the case may be, at a greater distance from the eye.

Finally, we think and speak of the visual image as magnified, or minified, according as the object under examination is represented upon the retina on a larger or smaller scale than that specified above.

5. Quality of the image furnished by the eye.

In connexion with the quality of the image furnished by the eye, or any other optical instrument, we have to consider in each case the question as to whether the image is satisfactory from the point of view of resolution in plan, resolution in depth, and freedom from *obfuscation*—meaning here and elsewhere by *obfuscation* the clouding of the retinal field by shadows projected upon it by intruding obstacles.

6. Resolving power of the normal eye.

Premising that we may, in dealing here with resolving power, conveniently confine our attention to the power of resolution in plan ; and premising, further, that resolution in plan may be conveniently measured in terms of the number of lines in the unit of measure which can be separately discriminated ; the following will be found to hold good with respect to the resolving power of the normal eye. Under the most favourable circumstances of illumination, lines ruled 8 to the millimetre (200 to the inch) and disposed at the near point of vision (250 mm., or 10 inches) can be glimpsed as separate. Lines ruled 4 to the millimetre, disposed at 250 mm. ; and lines ruled 1 to the millimetre disposed at 1 metre from the eye, can be discriminated with sufficient certainty to be separately identified.

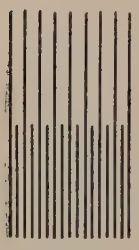


Fig 56.

Experiment 1. Take in hand the diffraction grating, ruled with 400 lines to the inch, supplied in the pocket of the cover of this book. Note that it cannot be resolved by the unaided eye.

Experiment 2. Turning to Fig. 56 where the lines are ruled in the upper part of the field 2 mm. apart, and in the lower half of the field 1 mm. apart, set up the book first at a distance of 1 metre, and afterwards at a distance of 2 metres from the eye.

Note that in the first case the lower rulings, and in the second case the upper rulings, can just be resolved with sufficient clearness to allow of separate identification.

7. Factors upon which the quality of the image depends.

The quality of the retinal image will depend upon two factors :—

(*a*) Upon the " optical quality " of the particular eye—meaning here by " optical quality " the figuring of the refracting surfaces, the adjustment of the depth of the eye to the focal length of the system, and the transparency of the ocular media.

(*b*) Upon the numerical aperture of the eye.

As compared with the N.A. of the eye, the factors enumerated under (*a*) exercise an altogether preponderating importance in connexion with the quality of the retinal image. In conformity with this, the various "errors of refraction" are very fully considered in the ordinary text-books of ophthalmology, while the question of the N.A. of the eye is hardly adverted to.

Our policy here must be a different one. We may conveniently leave out of consideration, as a subject-matter not directly related to the microscope, the effect that will be exerted on the retinal image by defects in the optical quality of the eye, and may, for a reason which will presently appear, consider the effect on the retinal image of the alteration of the numerical aperture of the eye.

8. Numerical aperture of the eye.

Where we are dealing (*a*) with the unrestricted normal pupil, i.e. with a pupil having a semi-diameter of 1·5 mm., (*b*) with a beam which completely fills it, and (*c*) with an emmetropic eye adjusted for the near point of vision (which is positioned at 250 mm. or 10 inches from the eye) the numerical aperture of the opening angle (N.A.) corresponds to $\frac{1{\cdot}5}{250} = 0{\cdot}006$.

The numerical aperture of the closing angle (n.a.) may be calculated either for the beam conceived as projected upon a receiving screen set up 250 mm. in front of the eye ; or—but this is less convenient, inasmuch as it involves taking into consideration the refractive index of the vitreous humour in which the image is formed —for the beam which actually comes to focus upon the retina.

Calculated upon the former basis, the numerical aperture of the closing limb (n.a.) would work out as 0·006, i.e. as exactly the same as the numerical aperture of the opening limb (N.A.).

Calculated upon the second basis, the numerical aperture of the closing limb (n.a.) would (taking the diameter of the normal pupil as 3 mm. and the depth of the normal eye as 20 mm.) work out as $\frac{1{\cdot}5 \times 1{\cdot}3}{20} = 0{\cdot}1$ nearly.

9. On the effect exerted upon the retinal image by a reduction of the numerical aperture of the eye.

It will be clear, on calling to mind the principles which have been set forth in the foregoing chapters, that any reduction in the

numerical aperture of the eye will impair the retinal image. As the aperture of the pupil is closed down, the image will suffer in the following respects :—

(1) Its luminosity will be progressively reduced until all distinction between light and shade is lost.

(2) Resolution in depth in monocular vision, depending as it does upon discrimination of the focussed from the unfocussed image, will be progressively impaired.

(3) The shadow of any obstacle intervening between the object and the eye will be carried back on to the retina in such a manner as to cut out from the image any element in the picture which lies wholly behind the obstacle.

(4) The retinal field will be more and more clouded by the shadows derived from extra- and intra-ocular obstacles, until it becomes, by reason of the obfuscation, impossible to see anything clearly.

(5) The antipoint will grow larger and larger, and such antipoint will, to the extent to which it is conspicuous, interfere with resolution in plan.

The following experiments will serve to bring these several effects clearly before the reader :—

Experiment 1. Make in a card a series of graduated pin-holes. Superposing upon each of these openings in turn the diffraction grating (ruled 400 lines to the inch, 16 to the mm.) provided in the pocket of the cover, read off with a pocket lens the number of divisions covered. This will give the measure of the several openings.

View now through the series of openings a printed page placed at a convenient reading distance in bright light.

Note as you pass from the larger to the smaller openings the progressive diminution in the luminosity of the image.

Experiment 2. Taking a cardboard box such as might serve for sending a bottle through the post, break out its top and bottom, and carry a threaded needle across its lumen in such a way as to form at some distance below the top of the box a grating of threads all running side by side in one direction, and at a lower level in the box another grating of threads all running at right angles to the first set.

Now hold the box up to the light and view the threads—using only one eye—first with the unrestricted pupil and then through one of the narrow pin-point openings.

In the first case it will be practicable by focussing in turn upon each system of threads and by attending to the difference in the sharpness of the focussed and unfocussed threads to discriminate nearer from farther.

In the second case it will be impossible to distinguish between the focussed and the unfocussed threads, and these, though situated on

different focal planes, will all coalesce in the image to form a grille of intersecting threads.

Experiment 3. Take a box fitted up exactly as in *Experiment* 2, except only in the respect that the threads at the two different levels shall all run in the same direction. View these threads through a slit aperture such as would be furnished by parallel rulers brought together so as to furnish a narrow chink.

It will now, in the case where the slit aperture is disposed at right angles to the direction of the threads, as in the case where the pupil is unobstructed, be possible to discriminate nearer from farther. This distinction will be lost in the case where the slit aperture is disposed parallel to the threads.

Experiment 4. Take before the eye one of the smaller perforations in the card and view through it a printed page placed at convenient reading distance in bright light.

Now dispose a needle in the neighbourhood of the pin-point aperture in such a manner as to intervene between the print and the retina.

A dark wedge of shadow will now be carried backwards from the needle on to the retinal image. This band of shadow will, as in Fig. 57, cut out from the imaged text one or more of the letters.

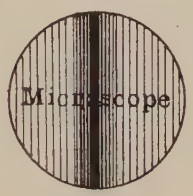


Fig. 57.

Retaining everything else in place, take a larger aperture before the eye and finally remove altogether the restricting aperture, the shadow will in the former case become fainter, and will in the latter case quite vanish from the retinal image, and the deleted letter or letters will be reinstated in the text.

Experiment 5. Replace the pin-hole aperture employed in the last experiment by a slit aperture furnished by parallel rulers brought together so as to form a very narrow chink.

When the narrow diameter of this slit opening coincides with the long diameter of the needle, the view will be obstructed exactly as in the case where the pin-hole opening is employed.

When the long diameter of the slit is disposed at right angles to the needle, this last will no longer cut out any of the letters.

Experiment 6. Look through the series of progressively smaller openings at the sky or any other blank, brightly illuminated, surface. Note that when the aperture of the pupil is cut down below 0·5 mm. the retinal screen becomes blotched with shadows ; and moving specks [1]—so called *muscae volitantes*—now course from time to time over the field.

Again, when the eyelids are half closed in such a manner as to bring the eyelashes across the pupil, note that these lashes cast dark shadows, blotting out from the picture elements which would, if the aperture

[1] These are seen also independently of any artificial restriction of the pupil when on gazing at the brightly illuminated sky the pupils contract spontaneously in response to the stimulus of bright light.

of the pupil had been unrestricted, in spite of intervening eyelashes, have been duly imaged by the agency of circumventing rays.

Experiment 7. Substitute for the perforated card employed in *Experiment* 6 a narrow slit-opening obtained by the aid of parallel rulers. Note that when the long axis of the slit is disposed parallel to the long axis of the eye-lashes these are brought into view. When by the disposal of the slit in the horizontal direction the pupil is left unrestricted along that meridian the eye-lashes are as inconspicuous as in the case of ordinary vision.

Experiment 8. Dispose a system of lines in bright light in front of the eye at such a distance as to give an image which can just be satisfactorily resolved. The finer rulings in the lower half Fig. 55 will comply with these conditions if they are set up in good illumination at a distance of 1 metre from the eye.

Taking now before the eye the perforated card, view the lines through the different pin-hole openings, beginning with the larger and going on to the smaller.

Note that when the aperture of the pupil is reduced to 0·75 mm. the lines begin to be sensibly thickened. When the aperture of the pupil has been reduced to 0·5 mm. the progressive thickening of the lines—which can be conveniently followed in the upper half of the field—has advanced to the point at which resolution of the finer rulings is definitely lost.

10. Significance of the results arrived at in the foregoing experiments, in relation to the question of the limitation of resolution by the aperture of the objective.

The significance of the results arrived at in the foregoing experiments, in relation to the question of the resolution of the microscopic image, is not immediately apparent, inasmuch as the aperture of the pupil is not, in the case where we view objects through the microscope, artificially restricted by taking a pin-hole before the eye.

Reflection on the facts set forth in *Cap.* VII, *subsect.* 41, will, however, reveal a connexion.

It will become clear that the microscope is a piece of apparatus which cuts down the aperture of the beam to the extent to which it magnifies the image. As a result, the beam which enters the eye is, in the case where the high powers of the microscope are employed, cut down in much the same way as when a small pin-hole opening is disposed in front of the eye.

We shall, therefore, do well to note the teachings of the above experiments, with a view to applying them afterwards to the case where the aperture of the pupil is cut down by the employment of the high powers of the microscope.

The teaching of the foregoing experiments may be summarized as follows. Given the case of an object viewed from a distance of 250 mm.—

(1) The N.A. of the eye can be cut down from ·006 to 0·002 without producing any serious deterioration of the image.

(2) When the numerical aperture is cut down to 0·0015, the dimensions of the conspicuous antipoint will—given sufficiently brilliant illumination—be sensibly increased. There will as yet be little trouble from obfuscation, and little loss of resolution in depth

(3) When the numerical aperture of the eye is cut down to 0·001.

(*a*) Resolution in depth is seriously interfered with, and

(*b*) Obfuscation becomes so pronounced that it becomes difficult to discern clearly any feature in the picture.

(*c*) The dimensions of the conspicuous antipoint may be such as to abolish resolution.

11. Question of the existence of an absolute physical limit to the resolving power of the eye, irrespectively of such limitations as may be imposed by the structure of the retina.

We shall see in *Cap.* XVI that Helmholtz took up the position that, in any optical instrument, the dimensions of the antipoint must impose a final limit upon resolution—that limit being reached at or before the point at which adjoining false discs reciprocally overlap to such an extent as to bring in each case the edge of one false disc into the centre of the next.

We shall see (*Cap.* XVI, *subsect.* 2) that the limit of resolution would on this principle be furnished by the formula :—

$$\text{Limit of resolution} = \frac{0{\cdot}6\,\lambda}{\text{N.A.}}$$

It is accordingly of interest to ascertain where the formula of Helmholtz would place the final limit of resolution in the case of the restricted pupillary apertures which were in question above.

We may conveniently consider the concrete case of a system of lines set up at 1000 mm. from the eye, and of a pupil restricted to 0·75 and 0·5 mm. respectively so as to cut down the numerical aperture of the opening limb of the eye vista, in the first instance to 0·0004 *circ.*, and in the second instance to 0·00025.

Taking here as elsewhere $\lambda = 0{\cdot}0006$ mm., the limit of resolution would by the above formula in the two cases work out as follows :—

Limit of resolution for the unaided eye in the case where the N.A. of the beam entering the eye is 0·0004 is 0·00036 ÷ 0·0004 = 0·9 mm.

Limit of resolution for the unaided eye in the case where the N.A. of the beam entering the eye is 0·00025 is 0·00036 ÷ 0·00025 = 1·4 mm. *circ.*

It will be recognized that there is striking agreement between the results furnished by the formula of Helmholtz, and the fact that in *Experiment* 8, *subsect.* 9 *supra*, lines placed 1 mm. apart could no longer be resolved when the pupillary aperture was reduced to 0·5 mm.

12. Projection picture of the pupillary aperture and entoptic picture.

Neither the margin of the iris, nor the eyelashes, nor the tears on the front of the pupil, nor the opacities in the cornea lens and vitreous come into view on the retinal image in ordinary vision.

The fact that these do not throw shadows is accounted for, in the case of the extra- and intra-ocular opacities, by the large n.a. of the beams which are brought to focus on the retina, and in the case of the iris, by fact that light floods into the eye from all directions.

When these conditions no longer obtain, and when light streams into the eye from a single point, or small area of surface, in the form of a *homocentric beam*, i.e. an expanding cone of unfocussed light, the pupillary aperture is projected upon the retina in the form of a bright disc. Around this lies the shadow of the iris ; while upon the bright disc are silhouetted, as shown in Plate X, Fig. 4, tears, and corneal, lenticular and vitreous opacities ; further, in the case where the eyelids are half shut, eyelashes ; and lastly, in the case where the eyelids have just cleansed the front of the eye, a ridge of tears and debris swept up from the surface of the cornea (Plate X, Fig. 5).

Experiment 1. Having taken up a position in front of a bright light, bring a small pin-hole aperture in a card up close to one eye and cover up the other eye. The bright disc which is now in view corresponds to an unfocussed image of the pin-hole aperture. Having taken cognizance of the dimensions of the luminous disc, uncover the masked eye.

As the light now falls into the pupil of this eye the pupil of the observing eye will contract under the influence of a sympathetic reflex movement.

As it does so the disc image on the retina will become smaller.

When the eye is again masked the luminous disc will again expand.

The figure which illustrates *Experiment* 2 will make it clear that the effects which are observed are due to the cutting down and expansion of the cone of light as the pupil contracts and enlarges.

Experiment 2. Make two, or better three, pin-hole apertures in a card, disposing these about 1·5 mm. apart so as to fall when brought up close to the eye within the limits of the pupillary aperture.

Proceed as in *Experiment* 1.

When the light falls into the disengaged narrow eye the luminous discs in the observing eye will contract and the narrow band of shadow

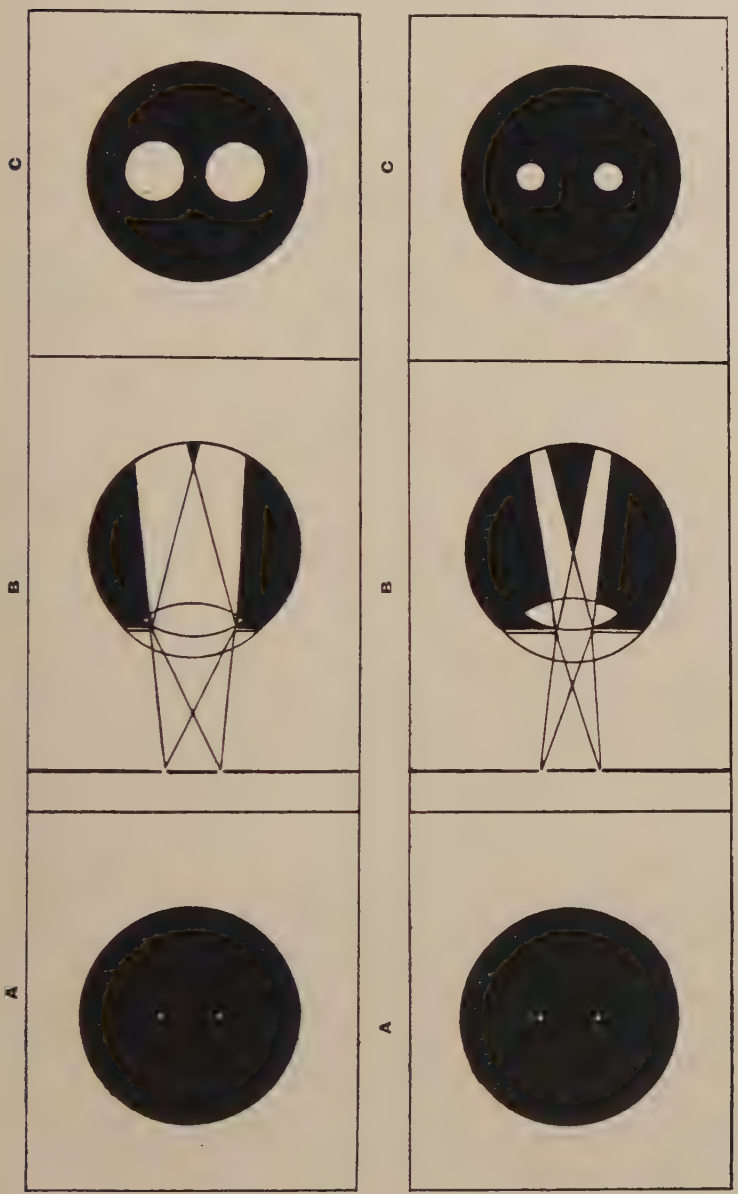


FIG. 58.

A. Pin-holes from which light falls upon the eye.

B. Optical section of the eye, showing that the beams are cut down by the pupillary margin of the iris.

C. Diagrammatic representation of the projection pictures of the pupillary aperture as developed on the retina.

between them will widen into a broad band. When the disengaged eye is again masked the discs will increase in size and the intervening band of shadow will shrink.

Experiment 3. Take in hand a pocket lens, or better a microscopic objective, or, better still, a glass spherule such as those employed in *Cap.* II in connexion with the study of the object picture. Hold it up in such a way as to form a minified image of a lamp disposed in front of any dark background, e.g. the dark background of a window at night. Now bring the eye close up to this aerial image.

A bright disc similar to that seen in the previous experiments will now be in view. The bright disc in question is, as before, the projection of the pupillary aperture. The shadows upon the disc are the magnified shadows of tears and opacities in the cornea and vitreous. Note that these are enveloped in diffraction rings.

Proceed now as in *Experiments* 1 and 2. The disc will, as before, contract and enlarge as the movements of the pupil cut down and open up the cone of light which radiates into the pupil from the luminous surface which corresponds to the minified image of the light source.

Now half close the eyelids and watch the shadows of the eyelashes coming into view, each eyelash being represented in the image on a magnified scale with a diffraction band[1] on each edge (Plate X, Fig. 5). Bring now the eyelids together for an instant as you are observing the disc. As you open them again, the shadow of a ridge of tears and débris which has been swept up by the movements of the eyelids will come into view.[2]

[1] These diffraction phenomena, like those referred to above, are more particularly conspicuous when the radiant point is carried away to some little distance from the eye.

[2] All the phenomena which have been described in this experiment are continually obtruding themselves on the observation of those who wear spectacles when light is projected into the eye from the rim of the spectacle-frame. They force themselves also upon the attention in a very striking manner when, on a rainy night in the open, rain drops collect on the spectacles and focus the street lamps close up to the aperture of the pupil in such a manner as to send a homocentric beam into the eye.

The reader who is interested in the history of the study of entoptic vision and in the practical applications of the method may with advantage consult the very interesting papers of Prof. W. F. Barrett, F.R.S., in the *Scientific Proceedings* of the Royal Dublin Society, Vol. XI (N.S.)., Nos. 7 and 8, March and May, 1906.

CHAPTER XI.

IMAGE FORMATION IN THE CASE WHERE AN OBJECT IS VIEWED THROUGH A SIMPLE MICROSCOPE.

Introductory—Inversion—Disposition of the reinforcing lens in the vista—Magnification—Methods of measuring the scale of the image obtained in the case where a convex lens is used as a doublet with the optical system of the eye—Description of the eikonometer and procedure for measuring the magnifying power of a simple microscope by its aid—Quality of the image—Effect of artificially shutting down the numerical aperture of the simple microscope—Delimitation of the field of the image.

1. Introductory.

In the optical arrangement known as that of the simple microscope we have, as in the case of unaided vision, a single vista. In this vista we have a convex lens or combination of lenses working as a doublet with the optical system of the eye.

2. Inversion.

In accordance with the fact that we are dealing, in the case of the simple microscope, with a single vista, the image which is formed upon the retinal screen is inverted as shown in Fig. 59 B. We have seen that an inverted retinal image is interpreted by the mind of the observer as an erect image.

3. Position of the reinforcing lens in the vista.

The position of the lens which is employed to reinforce the optical system of the eye may be varied at pleasure.

The largest magnification is obtained when the lens is disposed at its full focal length from the object. The largest field is, as we shall see, obtained when the lens is brought close up to the eye.

4. Magnification.

The image which is formed on the retina by the aid of a convex lens working as a doublet with the optical system of the eye is magnified—as compared with that obtained in the case of the unaided eye—to the extent to which the opening limb of the vista has been shortened.

In the case represented in the figure below, the opening limb of the vista measures 25 mm., in lieu of 250 mm. as in the case of unaided vision. We have here, accordingly, an image on ten-fold magnified scale.

5. Methods of measuring the scale of the image obtained in the case where a convex lens is used as a doublet with the optical system of the eye.

The scale of the magnification obtained in the case where a convex lens forms a doublet with the optical system of the eye may be arrived at by any of the following methods :—

(1) We can measure the opening limb of the vista and arrive at the magnification by dividing this length expressed in millimetres into 250, the number of millimetres contained in the opening limb of the vista in the case where an object placed at the near point of vision is viewed by the unaided eye.

The measurement which is here required is a somewhat difficult one to make in the case where we are dealing with a lens combination or with a lens of short focal length.

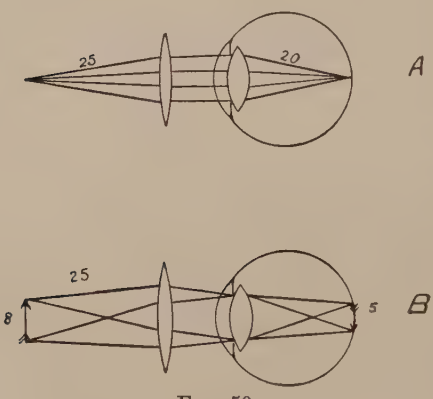


Fig. 59.

(2) We can, viewing with one eye through the lens a scale, and viewing with the other eye without the assistance of a lens another scale placed at 250 mm. from the eye, by a mental effort combine the two images and compare their dimensions.

(3) We can, viewing with one eye the image of a scale seen through the lens, and with the same eye the reflected image of a similar scale placed a distance of 250 mm. and seen without the intervention of the lens, directly compare their dimensions.

In addition to involving the use of apparatus which is somewhat difficult to adjust, this method will be found to involve—as does also Method 2—a careful balancing of the brightness of the magnified and unmagnified images which are being compared.

(4) We can, by the exploitation of a fiduciary phenomenon furnished by a diffraction grating, select, from a series of paired rulings, two pairs of rulings which give, the one when

viewed by the unaided eye, and the other when viewed by the lens-armed eye, in each case a retinal image of exactly similar dimensions.

The quotient obtained by dividing the elongation of the first-mentioned by the elongation of the second-mentioned pair of lines, corresponds to the magnifying power of the lens.

The details of the method of procedure have been already furnished in *Cap.* IX, *Appendix* 2.

(5) We can—and here, as in Method 4, we make a new departure—place behind the lens whose magnifying power is to be elicited a focussing lens, and again behind this in its principal focal plane a micrometrical scale.

In the case where the focal length of the focussing lens conforms to the focal length of the eye, the image obtained upon the screen which carries the micrometrical scale will manifestly conform to the image formed upon the retina.

Where, again, the focussing lens possesses a focal length of 250 mm., the image obtained will correspond to the image which is projected outwards from the retina upon an image plane disposed 250 mm. from the eye.

And again, when the focal length of the focussing lens represents an aliquot fraction of 250 mm., the image formed upon the micrometrical scale will be smaller than the image projected from the eye to the near point of vision in the proportion in which the principal focal length of the focussing lens is less than 250 mm.

The author's eikonometer, which is figured below, is constructed upon the optical principles just set forth.

6. Description of the eikonometer[1] and procedure for measuring the magnifying power of a simple microscope by its aid.

The eikonometer represented in perspective and in section in the figures below consists essentially of two optical elements.

The first optical element is a plano-convex lens of 25 mm. focal length. This focusses upon the micrometrical scale placed in its principal focal plane the pencils of parallel rays which are furnished by any magnifying lens that is working at its principal focal distance from the object.

The micrometrical scale is ruled in divisions one-tenth of a millimetre apart. The value of these divisions is equivalent to that

[1] Described *Proceedings Royal Microscopical Society*, 1904.

of millimetre divisions disposed in the principal focal plane of a focussing lens possessing a focal length of 250 mm.

The second optical element in the eikonometer is a Ramsden eye-piece. Here a combination is employed which has a focal length of 25 mm. and a corresponding magnifying power of 10 diameters.

The Ramsden eye-piece focusses the scale upon the observer's retina and, exactly compensating for the minification effected by

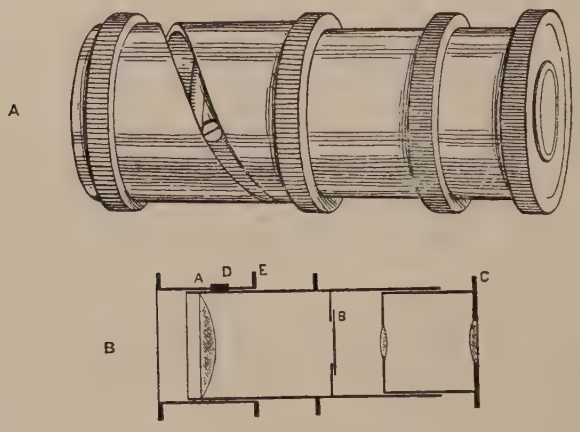


FIG. 60 (A AND B).

EIKONOMETER—A, PERSPECTIVE VIEW; B, OPTICAL SECTION.

A, focussing lens (corresponding to the lens system of the eye); B, receiving screen (corresponding to retina) carrying a micrometrical scale ruled in tenths of a millimetre; C, eye-piece focussing upon the micrometrical scale; D, stud fitting into a spiral slot in E, outer sleeve, which allows of the eikonometer being raised or lowered in such a manner as to bring the focussing line into the plane of the Ramsden disc of the lens whose magnifying power is being measured.

the focussing lens of the eikonometer, gives to the one-tenth mm. divisions of the micrometer scale the value of millimetre divisions placed at a distance of 250 mm. from the eye.

In addition to the focussing arrangement for the Ramsden eye-piece, there is provided also an arrangement which allows of the focussing lens being disposed accurately in the Ramsden disc of the lens under examination, in such a manner as to take in the whole of the field.

In the arrangement adopted the tube of the eikonometer moves up and down in an outer sleeve—the movement being regulated by a stud fitting into a spiral slot.

Instructions for measuring by the aid of the eikonometer the magnifying power of the simple microscope.—Place on the table a millimetre scale and dispose the lens whose magnifying power is to be measured at its principal focal distance from the scale in such a manner as to

obtain the largest possible image. Now place the eikonometer on the top of the lens and read off the number of divisions of the eikonometer scale covered by the image of a division of the object scale. The number of divisions covered gives the magnifying power of the lens.

7. Quality of the image.

If the eye behind the simple microscope is in all respects normal the quality of the image obtained will be dependent upon the accurate figuring and correction of the lens combination and the numerical aperture of the beam which passes through the simple microscope into the eye.

BATTERSEA PUBLIC LIBRARY

Resolution in plan.—In the case where the figuring and the correction of the simple microscope is to all intents and purposes perfect, the resolution obtained will (assuming that the pupil is not artificially restricted in such a manner as to reduce the *n.a.* and thereby to increase the dimensions of the antipoint) be superior to the resolution obtained with the unaided eye in the proportion in which the scale of the image obtained with the simple microscope is greater than the scale of the image obtained with the unaided eye.

Resolution in depth.—The numerical aperture of the opening limb of the vista in the case where the simple microscope is employed is to the numerical aperture of the vista in the case where the unaided eye is employed inversely as the focal length of the simple microscope is to the focal length of the eye when focussed upon the near point of vision. In correspondence with this resolution in depth will be improved in the image obtained with the simple microscope as compared with the image obtained with the unaided eye.

Obfuscation.—We have seen that the image becomes clouded with shadows in the case where the numerical aperture of the closing limb of the vista is reduced. No such restriction of the numerical aperture of the closing limb of the vista occurring when a simple microscope is taken before the eye, it follows that the retinal image which is furnished by the simple microscope working in conjunction with the eye will be as little troubled by shadows as the image furnished by the unaided eye.

Experiment 1. Take before the eye a convex lens which gives a fourfold magnified image when working at its principal focal distance from the object. View through this lens the diffraction grating supplied with this book, and note that this grating, which is ruled with sixteen lines to the mm., is as well resolved as lines $\frac{1}{4}$ mm. apart viewed by the unaided eye from a distance of 250 mm., or lines 1 mm. apart viewed from a distance of 1 metre.

It will be remembered that the standard of resolution for the normal eye is four lines to the millimetre at a distance of 250 mm., or one line to the millimetre at a distance of 1 metre.

Experiment 2. Inscribe with ink or a glass-writing pencil upon each of two cover-glasses a system of parallel rulings. Superpose the cover-glasses one upon another in such a manner that the ruled surface of each cover-glass may be uppermost, and dispose the cover-glasses so that the lines may intersect in such a manner as to form a pattern of squares. Now cover in with another cover-glass.

Looking down upon the cover-glasses from above, it will be difficult to tell that the rulings are disposed upon separate optical planes. Taking now a convex lens in front of the eye, bend down over the object until a clearly focussed image is formed upon the retina. The fact that the two systems of rulings are disposed upon separate optical planes will now be apparent in the fact that when the one system of rulings is seen in accurate focus the other will be out of focus.

8. Effect of artificially shutting down the numerical aperture of the simple microscope.

The effect of shutting down the numerical aperture of the simple microscope by graduated pin-hole openings placed either before or behind it will be similar in all respects to the effect exerted by such pin-holes upon the image furnished by the unaided eye.

Experiment 1. Taking in hand the lens employed in *Experiment* 1 *subsect.* 7, shut down its aperture by the aid of the perforated card employed in *Cap.* X, *subsect.* 9, and view in succession through the series of pin-holes the diffraction grating supplied with this book. Note that the image is not seriously impaired and, in particular, that resolution in plan is not abolished until the numerical aperture of the closing limb of the vista is reduced to 0·001.

Experiment 2. Stopping down in the same manner as before, the aperture of the lens, and viewing through it a brightly illuminated blank surface, note that the aperture of the closing limb cannot be reduced to 0·001 without producing serious obfuscation.

Experiment 3. Placing before you the same arrangement of ruled cover-glasses as in *Experiment* 2 in *subsect.* 7, and stopping down the lens as above, note that both systems of lines are brought into view at one and the same time, constituting, as in the case when they are viewed by the unaided eye, a system of squares.

9. Delimitation of the field of the image.

It has already been laid down above (*subsect.* 3) that the extent of the field which is imaged by the simple microscope is greatest when the lens is brought quite close up to the eye, and that it diminishes progressively as the lens is carried away from the eye.

The reason of this will appear on comparing Fig. 61, below, with Fig. 59, B.

It will be seen that in Fig. 59, B, the beams which proceed from the object all enter the eye and are focussed upon the retina.

In Fig. 61, on the contrary, those from the periphery of the field go wide, with the result that the field of view is restricted.

In point of fact, the field is in this case cut down by the pupillary margin of the pupil.

The condition of affairs under the two contrasted conditions will be further elucidated by the following experiments.

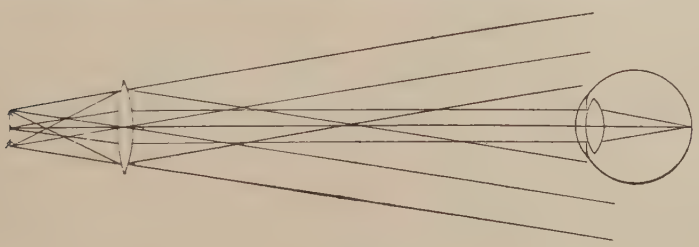


Fig. 61.

Experiment 1. Bring a pocket lens up close to the eye, and bend down over this page, or over other printed matter, until the text just comes into view in accurate focus. Count the number of letters included in the field.

Maintaining the lens in position, carry the eye farther and farther away. Note that the magnification remains what it was, while the field of the image is progressively restricted.

Experiment 2. Placing any printed matter upon the stage of the microscope, focus down upon it with the low power of the microscope, having first discarded the ocular and having placed the eye at the open upper end of the barrel of the microscope.

Now shut and open the disengaged eye. As you do so note that the field of view expands and contracts, showing that it is now, as in *Cap.* X, *subsect.* 11, delimited by the pupillary margin of the iris.

Experiment 3. Take in hand a microscopic ocular. Unscrewing the field lens, insert into it from below a disc of printed paper. This done, hold the eye-piece up to the light and look into the eye lens, placing the eye in the ordinary way close to the lens, in such a position that its Ramsden disc occupies the pupil. Note that the field of view is now delimited by the margin of the diaphragm of the eye-piece.

Close and open the unengaged eye and observe that this makes no difference to the extent of the field of view.

Now carry the eye gradually away from the eye-piece, and note that first the edge of the diaphragm, and then zone after zone of the peripheral field, is lost to view.

Close and open the unengaged eye and observe that the field is now limited by the pupillary margin of the iris.

CHAPTER XII.

IMAGE FORMATION IN THE CASE WHERE THE OBJECT IS VIEWED THROUGH THE COMPOUND MICROSCOPE.

Introductory—Optical system of the compound microscope—Detailed consideration of the microscope stage and eye-piece diaphragm (objective and field lens) vista—Magnification achieved in this vista and procedure for measuring this magnification—Measurement of the extent of object field which is imaged—Quality of the image furnished by the objective—Eye lens and eye vista—Magnification achieved in this vista and methods for the measurement of this magnification—Quality of the image furnished by this vista—Vista of the sub-stage condenser—Scale and quality of the image furnished by the condenser—Catena of the apertural planes of the microscope—Configuration and course of the beams in the catena in question—Scale of the image furnished in the Ramsden disc of the eye lens—On the primary catena of the microscope considered as a whole and on the terminal image which is furnished by this catena—Procedure for measuring the magnifying power of the microscope by means of the eikonometer—Procedure for measuring the magnifying power of the microscope by the aid of a stage micrometer and diffraction grating—Procedure for arriving at the magnification by dividing the numerical aperture of the opening limb of the objective and field-lens vista by the numerical aperture of the closing limb of the eye lens and eye vista—Quality of the terminal image, and on the superimposition upon this of an entoptic picture.

1. Introductory.

The essential distinction between the simple and the compound microscope is to be found in the circumstance that we are dealing in the former with a single magnifying vista, in the latter with a catena of magnifying vistas.

An understanding of the more complicated optical arrangements which are here involved will be arrived at in the simplest manner if we commence by the following experiments.

Experiment 1. Placing a printed page before you at arm's length, take in hand a pocket lens, or a low-power microscopic objective. Begin by placing the lens close to the print and then gradually withdraw it.

The following will be observed :—

(*a*) As long as the lens transmits pencils of slightly divergent or parallel rays—as long, in other words, as the lens is working within its

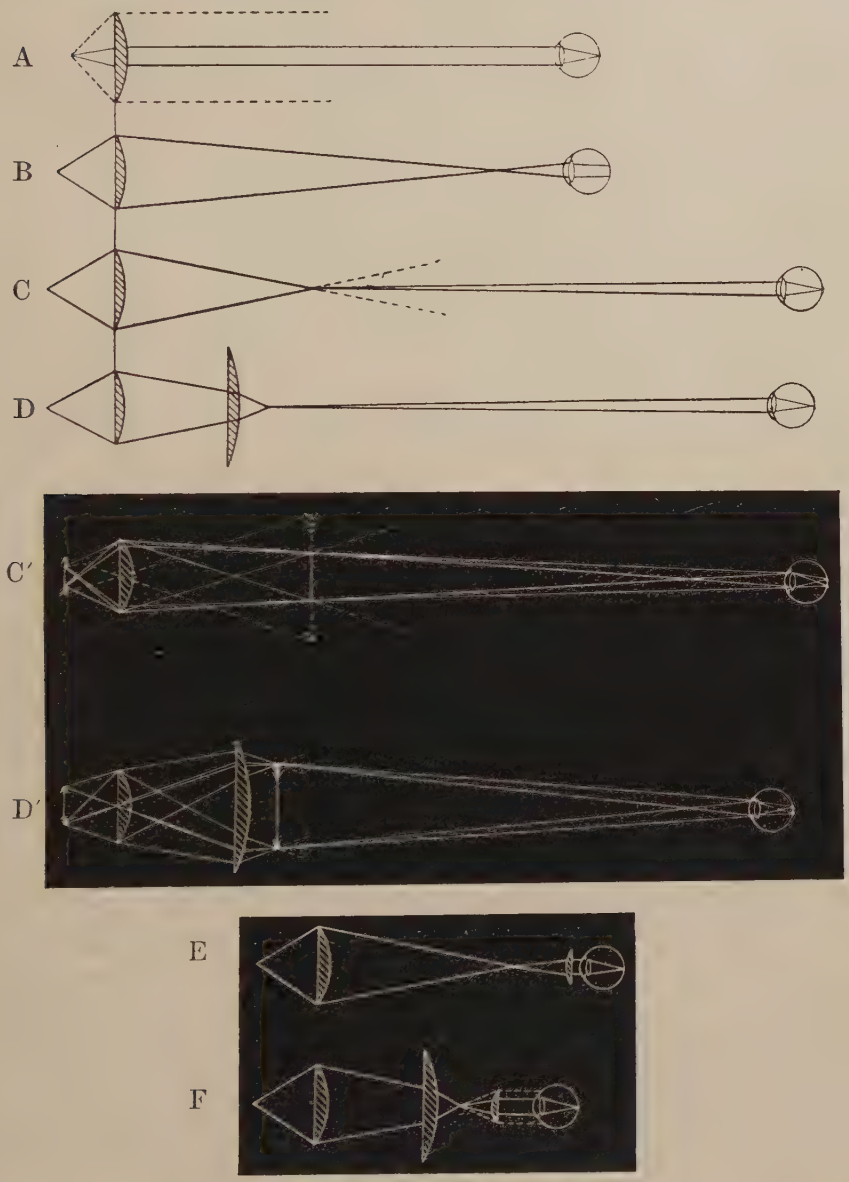


Fig. 62.

focal distance and is functioning as a doublet with the optical system of the eye—the print will be imaged upon the retina in the inverted position and will be interpreted as erect.

The optical arrangement here in question is of course that of the simple microscope (Fig. 62, A).

Experiment 2. Withdraw the lens beyond its focal distance. The light will now emerge from its posterior surface in the form of pencils of convergent rays, which will come to focus somewhere in front of the eye, but too close to it to be refocussed upon the retina (Fig. 62, B).

The field of view will at this stage be occupied only by diffusion discs and the optical arrangement will correspond to a completed followed by an incompleted vista.

Experiment 3. Withdraw the lens still farther from the print, or (and this of course amounts to the same) carry back the eye until the aerial image which has been formed by the lens lies outside the near point of vision. The image will now be refocussed upon the retina by the optical system of the eye. The image thus formed will be erect. It will be interpreted as inverted,and the optical arrangement will correspond to a catena of two vistas (Fig. 62, C and C′).

Experiment 4. Re-constituting the arrangement in *Experiment* 3, *supra*, take into the disengaged hand another convex lens and interpose it between the first lens, which is in such an arrangement technically denoted the objective, and the aerial image. As a result of the introduction of this " field lens " the field of the image will be extended, the scale of magnification being proportionately reduced.

The optical arrangement will now be that shown in Fig. 62, D and D′.

It is to be noted that the effect exerted by the field lens increases and diminishes as this lens is, as the case may be, brought nearer to or carried farther away from the objective.

Experiment 5. Maintaining the arrangement in *Experiment* 3, *supra*, and taking again into the unoccupied hand a convex lens, bring this close up to the eye, and now bend down over the aerial image furnished by the objective until this comes clearly into view.

The optical arrangement is now that shown in Fig. 62, E.

Experiment 6. Fixing the objective in position, take into each hand a convex lens. Introduce one of these as in *Experiment* 4, *supra*, between the objective and the aerial image, the other, as in *Experiment* 5, *supra*, between the aerial image and the eye.

The optical arrangement is now that shown in Fig. 62, F.

This arrangement corresponds, as we shall see in the next section, in all essential points with that employed in the microscope.

2. Optical system of the compound microscope.

It has clearly appeared in the foregoing that we have in the case of the microscope to deal with a catena of two magnifying vistas. We may conveniently distinguish each of these vistas by a name. The first, or opening vista, we may, according as we have in view (*a*) the positions of the plane of origin and terminal plane of the vista, (*b*) the lens systems which come into consideration, or (*c*) the more important of these, speak of as the *microscope*

stage and eye-piece diaphragm vista, or the *objective and field-lens vista*, or simply the *objective vista*. The second and terminal vista we may on similar grounds speak of as the *eye-piece diaphragm and retina vista*, or, alternatively, the *eye lens and eye vista*, or simply the *eye-lens vista*.

In the case where the microscope is furnished with a substage condenser a third vista is concatenated to the vistas already spoken of. We may speak of this third vista as the *light source and microscope stage*, or alternatively as the *condenser vista*.

We may denote the succession of three vistas, *the primary catena of the microscope*.

It will be profitable to rehearse again, in connexion with the microscope, the experiments undertaken in the previous section.

Experiment 1. Place upon the stage of the microscope any printed matter—by preference let it be matter printed in small type upon thin paper. Remove the eye-piece from the barrel of the microscope and focus down with a low-power objective until an erect image of the print is obtained.

The optical arrangement is now conformable to that in *Experiment* 1 of the last subsection. In other words, we have to deal with a single magnifying vista in which the objective is working at its principal focal length as a doublet with the eye.

Experiment 2. Now rack back the tube until the interval between the print upon the stage and the objective just exceeds its focal length. The light which before emerged from the back of the objective in the form of pencils of parallel rays now emerges from it in the form of pencils of converging rays coming to focus in the upper part of the barrel of the microscope, but too close to the observer's eye to produce a clear image on his retina. The optical arrangement will now be conformable to that in *Experiment* 2 of the last subsection.

Experiment 3. Withdraw the eye to a distance of 10 inches or more from the upper end of the barrel of the microscope. An inverted magnified image of the print upon the stage will now, as in *Experiment* 3 of the last subsection, be in view.

Experiment 4. Unscrew now the eye-lens and reinsert the ocular into the microscope after slipping round it a rubber ring to hold it up in its place. On looking down upon the aerial image from a distance of 10 inches or more, it will be seen that a more extended field and a proportionately smaller image will be obtained than in the absence of the field lens. (Compare in this connexion Fig. 62, C′ and D′ *supra*). The image will, of course, still be seen as inverted.

Experiment 5. Withdrawing the ocular, take in hand now the eye lens, hold it close up to the eye and bend down over the microscope tube until the aerial image formed in the upper end of the barrel of the microscope comes into view. The conditions will now be similar to those in *Experiment* 5 in the last subsection.

The image will, like that obtained in *Experiment* 3 of the present subsection, be seen as inverted. It will differ from it in the fact that it will be magnified by the power of the eye lens.

Experiment 6. Restoring the eye lens to its position in the ocular, and looking down through it (adjusting the position of the objective if this should be necessary) the conditions will be similar to those in *Experiment* 6 in the last subsection. The image obtained will be seen as inverted and will correspond to that in *Experiment* 4 above, magnified by the power of the eye lens.

Experiment 7. Substituting for the print upon the stage a transparent object or, in default of this, giving transparency to the printed paper by placing on it a drop of oil, focus the condenser upon the object in the manner prescribed in *Cap.* XIII, *subsect*, 4.

An image of the light source is now brought into view at the same time as the object on the stage of the microscope.

We have now concatenated the condenser vista with the objective and eye-lens vistas, and an image of the light source is now brought into view in association with the image of the object on the stage of the microscope and with the image of the diaphragm of the ocular which lies in the principal focal plane of the eye lens.

Let it be noted: (*a*) that the image of the light source is seen as erect. This is in conformity with the fact that it has undergone inversion in three successive vistas. (*b*) That the image of the object upon the stage is seen as inverted. This is in conformity with the fact that it has undergone inversion in two successive vistas, and (*c*) that the image of the eye-piece diaphragm is seen as erect.[1] This is in conformity with the fact that it has undergone only one inversion.

3. Detailed consideration of the microscope stage and eye-piece diaphragm (objective and field-lens) vista.

Contour of the vista and course of the beams. Fig. 63 shows the general contour of the vista and the course followed by the axial and most obliquely incident beams respectively.

Opening limb of the vista. The opening limb and the equator of the vista are shown in more detail in Plate VII, Fig. 3. It will be seen that the expanding beams (here coloured respectively red and blue) take origin from radiant points upon the microscope stage and fall upon the front lens of the objective combination (here shown for simplicity as consisting of two lenses only). Emerging from the back surface of the front lens with a reduced angle of divergence, they enter the posterior lens, intersect here, and are converted into pencils of parallel rays which finally, on emergence from the lens, become converted into pencils of convergent rays.

[1] This can readily be verified by inscribing a fiduciary mark upon the upper or lower margin of the diaphragm of the eye-piece.

Apertural plane. The optical features of the apertural plane are also exhibited in Plate VII, Fig. 3. We have here represented three beams, coloured respectively red and blue, passing through the objective. The figure makes clear that these beams intersect in the neighbourhood of the back surface of the posterior lens of the combination, and that they form here a system of nodal points which radiate (through an angle which corresponds to the field angle) in each case both blue and red light.

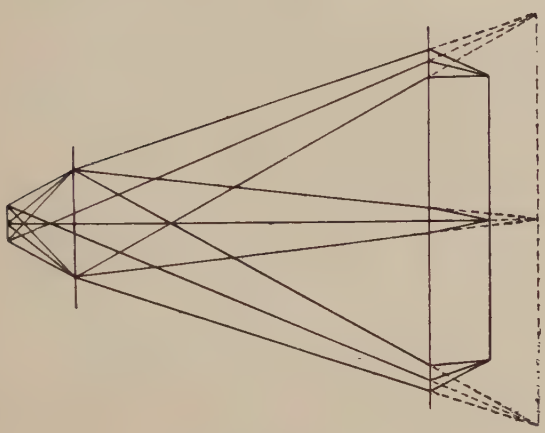


Fig. 63.

Experiment 1. Using a blue pencil, inscribe upon a piece of white paper a blue disc of such a size as to occupy about two-thirds of the field of a low-power objective. Using a red pencil, now encircle this blue disc with a red annulus.

Taking the low-power objective in question before the eye, bend down over the figure until an erect image is obtained. Now withdraw the objective until the object becomes blurred. It is now working as in the microscope beyond its principal focal distance. Fixing the objective in this position bring into view under a pocket lens the optical plane which corresponds to the back of the posterior lens. It will be found that this optical plane radiates to the eye light of a uniform purplish colour. In point of fact the optical plane in view is now occupied by just such a system of radiant points as that depicted in the figure.

Experiment 2. Maintaining unaltered the optical arrangement in *Experiment* 1, drop upon the back surface of the objective a fine glass filament. As seen through the pocket lens this filament will appear as a blue object outlined in red upon a blue field.

The explanation of this appearance is furnished by the fact that the glass filament has, in the manner explained in *Cap. i, sect.* I *subsect.* 6 (*d*) in connexion with the glass spherule, analysed the purplish beams which proceed from the nodal points in the Ramsden disc into their blue and red components. (Vide in this connexion Plate I, Fig. D, Plate II, Figs. *h* and *m*, Plate IV, Fig. 1, and Plate XI, Figs. 1 and 2).

Closing limb of the vista. Emerging, as shown in Fig. 63, from the back of the objective combination as pencils of converging rays,

the beams would—were it not for the interposition of the field lens—come to focus in the manner indicated in Fig. 63 by the dotted lines.

Falling as they do upon the field lens, they are brought to focus as shown in the figure by the unbroken lines.

The field lens effects, as will be seen (*a*), a focussing of the beams upon a nearer focal plane, and (*b*) in the case of the beams which fall eccentrically upon this lens, also a bending in of the axes of these beams towards the centre of the field.

In conformity with these displacements, there is obtained in the diaphragm of the ocular a less magnified but proportionately more extensive view of the object upon the stage (cf. *Experiment* 4, *subsect.* 1, *Experiment* 4, *subsect.* 2, *supra*, and Figs. 62, D, and 63).

4. Magnification achieved in the objective vista and procedure for measuring this magnification.

Of the many methods which are available for the measurement of the magnification achieved in the objective vista the following is the simplest.

Procedure. Place upon the stage of the microscope a stage micrometer, or, in default of this, the ruling (16 lines to the mm.), which is supplied with this book for the purposes of diffraction experiments. Focus down upon the ruling with a low-power objective. Read off the number of divisions of the micrometer which are included in the field. This reading will give us the diameter of the field which is imaged by the microscope.

This done, unscrew the lenses of the ocular, insert into its vacant tube a disc of paper and press it home against the diaphragm. Now mark off upon this paper with the point of a pencil the dimensions of the diaphragm. On measuring this and dividing by the dimensions of the object field, we obtain the magnification achieved in the objective vista.

5. Measurement of the extent of object field which is imaged.

The measurement of the object field which is imaged can be obtained—(*a*) either, as was done in the last section, by placing a micrometer scale upon the stage and reading off the number of divisions which are brought into view.

Or alternatively (*b*) by measuring, as was done in the last section, the diameter of the diaphragm of the eyepiece and dividing by the magnification.

6. Quality of the image furnished on the terminal plane of the objective vista.

Leaving out of consideration questions relating to the figuring and corrections of the objective field-lens combination, we may here consider the question of the effect exerted on the quality of the

image by the widening, or, as the case may be, the closing down of the aperture of the opening limb of the objective vista.

The widening of the aperture of the opening limb improves the image by cutting down the length of the shadows cast by elements in the microscopical preparation which lie above or below the optical plane which is for the moment under observation.

Again, the widening of the opening angle has as its result a widening of the closing angle of the vista.

This in its turn improves the image, (*a*) by reducing the dimensions of the antipoint, and (*b*) by freeing the image from the shadows cast by intrusive obstacles in the closing limb—by such obstacles for instance as particles of dust upon the field lens.

The experiments which illustrate these points are reserved till we come, in *Cap.* XV, to deal with the adjustments required for obtaining a critical microscopic image.

7. Eye lens and eye vista.

Configuration and course of the beams. The general configuration of the vista and the course of the beams is set forth in Fig. 64.

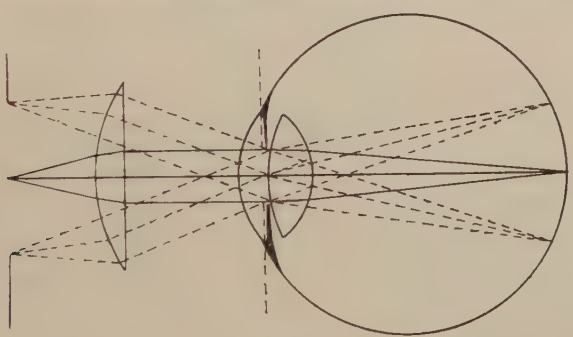


Fig. 64.

Opening limb of the vista. Taking their departure from the image plane of the objective and field-lens vista in the diaphragm of the eyepiece, the beams which are here in question expand until they fall upon the eye lens. They are by this lens converted into pencils of parallel rays. Of these pencils the central one, which lies in the axis of collimation of the instrument, pursues its course undeflected; the outlying ones which traverse the periphery of the lens are, as shown in the figure, thrown inwards by refraction in such a manner as to cross each other's path.

Apertural plane (*Ramsden disc*). The region upon which all the beams, which traverse, as shown in each case, different regions of the eye lens converge, is the Ramsden disc or apertural

plane of the eye-lens vista. Its appearance, as seen by an observer looking down upon the eye-lens of the microscope from a distance of 10 inches or more, is shown in Figs. 1 and 2, Plate X. Its appearance, as seen when examined with the aid of a pocket lens, is shown in Plate X, Fig. 3, A, B and C.

Its optical structure comes clearly before the eye on following out the course of the blue and red rays in Plate VII, Fig. 4. Proceeding from the image plane of the objective vista, these rays are seen to intersect in such a manner as to form an interfusion disc. From the nodal points here formed a mixture of red and blue light is radiated.

This system of points constitutes the counterpart (and, as we shall presently see, the image) of the system of radiant points which occupies the apertural plane of the objective (Plate VII, Fig. 3).

Experiment 1. Placing upon the stage of the microscope a parti-coloured radiant field such as that employed in the experiments in *subsec.* 3, *supra*, or projecting by means of the condenser an image of such a field upon the stage of the microscope, focus down upon it with a low-power objective. The Ramsden disc of the eye lens, examined with a pocket lens, will now be found to radiate a uniform purple light.

Experiment 2. Maintaining the arrangement employed in the last experiment, bring a fine filament of glass into the Ramsden disc of the eye lens. It will, as in *Experiment* 2 *subsection* 3, *supra*, be seen as a blue object outlined in red upon a blue field.

Closing limb of the vista. Having passed through the Ramsden disc as through an aperture, the pencils of parallel rays, which constitute the separate beams, spread out in the form of an expanding cone.

In the case, illustrated in Fig. 64, where the Ramsden disc is positioned in the pupil, all these beams are received into the eye and are all brought to focus upon the retina, with the result that the whole of the picture which lies in the diaphragm of the eye piece is brought into view. If the eye were disposed, as in Plate VII, Fig. 4, at some little distance behind the Ramsden disc, the delimiting beams of the vista would go wide of the pupil, and the field of view would be restricted by the pupillary margin of the iris, as in *Cap.* X, *subsect.* 11, *Experiments* 1–3, and in *Cap.* XI, *subsect.* 9, *Experiment* 2.

8. Magnification achieved in the eye vista, and methods for the measurement of this magnification.

The magnification which is achieved in the eye lens and eye vista

Plate X.

Fig. 1.

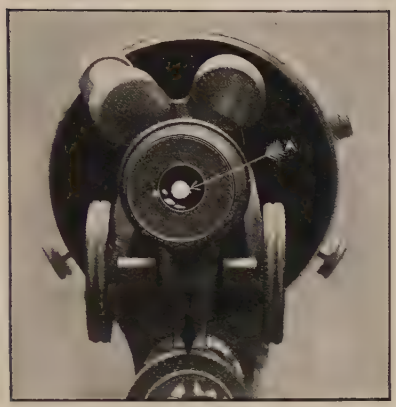


Fig. 2.

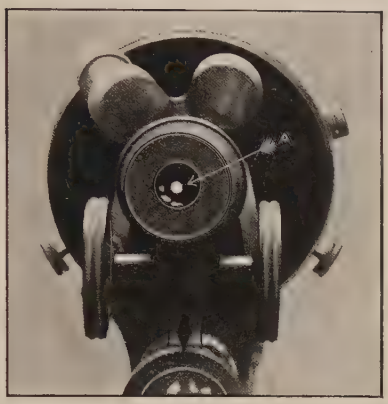


Fig. 3.

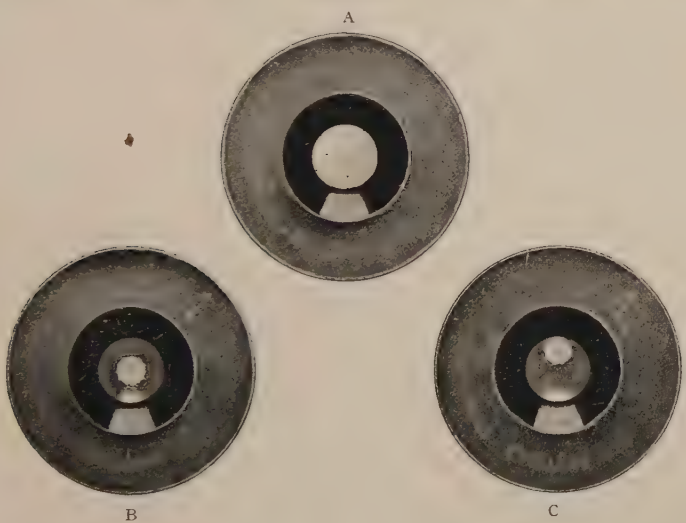


Fig. 4.

Fig. 5.

corresponds to the ratio in which the length of the opening limb of this vista stands to the length of the opening limb of the vista which comes into consideration when any object is viewed with the unaided eye from a distance of 250 mm.

This magnification may most conveniently be measured :—

(1) By measuring the diameter of the diaphragm of the eyepiece and reading off upon the scale of an eikonometer the dimensions of the image of the opening which is formed by the eye lens ; or, in default of an eikonometer,

(2) By unscrewing the eye lens and measuring the distance between eyepiece diaphragm and eye lens, and dividing this distance into 250 mm. or 10 inches.

9. Quality of the image furnished by the eye vista.

If we abstract the influence exerted by the figuring of the refracting surfaces of the eye lens and eye, the quality of the image will be determined by the aperture of the eye lens and eye vista, that is to say, by the dimensions of its Ramsden disc.

When the dimensions of that disc correspond to those of the normal aperture of the pupil, the image of the picture in the diaphragm of the eyepiece will be as little disturbed by diffraction phenomena and as little troubled by shadows of intra-ocular and extra-ocular intrusive obstacles as the images obtained in ordinary unaided vision. When the dimensions of the Ramsden disc correspond with those of a pin-hole of moderate dimensions, the disturbances which are due to obstructive interference and diffraction will be comparable to those experienced when we look at objects through such a pin-point opening. Finally, when the dimensions of the Ramsden disc correspond with those of the very finest point aperture, the disturbances caused by intrusive obstacles are, as will appear in *subsect.* 16 *supra*, such as to make anything in the nature of clear vision impossible.

10. Vista of the substage condenser.

Configuration of the vista. The configuration of the condenser vista will be seen on referring to any of the figures in *Cap.* XIII, *subsects.* 2–4. The radiant field which sends light to the mirror constitutes the plane of origin of the condenser vista. In the case where the sky furnishes the illumination, and where the plane mirror is employed—and this is the most convenient case to consider —the apertural plane of the vista is positioned in the substage

diaphragm. Here the pencils of parallel rays which have been reflected upwards from the mirror intersect and interfuse (Figs. 72, 73 and 74). The terminal plane of the vista is, in the case where the condenser has been duly focussed upon the plane of origin of the objective vista, positioned upon the stage of the microscope.

11. Scale and quality of the image furnished by the condenser.

The image of the light source which is obtained upon the terminal plane of the condenser vista is in each case a minified image. We are not, however, here concerned with the achievement of an image, *quâ* image. We are instead, as will appear in the next chapter, concerned with developing upon the optical plane occupied by the microscopic preparation a system of luminous points which shall be capable of radiating light into the objective in each case under the most appropriate angle. Such an angle will in many cases be the largest possible angle.

The circumstance that the condenser is designed to form upon the microscope stage a minified image will be recognized to stand in relation with the fact last referred to. We cannot, unless by the employment of a minifying vista, bring a beam to focus under a wide angle.

Before we rehearse and amplify, as we may usefully do, some of the points already adverted to in connexion with the vistas which constitute the primary catena of the microscope, we may conveniently turn aside and consider the catena of the apertural planes of the microscope.

12. Catena of the apertural planes of the microscope.

We have seen in *Cap.* VII, *subsect.* 36 that every optical plane in a catena is concatenated with a homologous plane in each succeeding vista, and with a homologous plane in each antecedent vista, and that the pattern of radiant points which occupies a particular optical plane in an antecedent vista is imaged in accurate focus in the homologous plane of the succeeding vista.

It follows that in the microscope the apertural plane of the objective vista will always be imaged in the Ramsden disc of the eye-lens vista ; further that in the case where the condenser vista has been concatenated with the objective vista the apertural plane of the condenser vista will be imaged in the apertural plane of the objective vista and re-imaged in the Ramsden disc of the eye-lens vista (Plate X, Fig. 3, A, B and C) ; lastly, that in the image of

the Ramsden disc of the eye lens which is formed upon the retina by the aid of a pocket lens, the apertural planes of the two antecedent vistas are brought into view.

Experiment. Set up a microscope in front of the window, or other convenient source of light. Fit to the tube a low-power objective; focus down upon a blank slide and then focus the condenser upon the slide in the manner described in *subsect.* 4 of the next chapter. Now narrow the substage diaphragm in such a manner as to transmit a beam which will fill in only a part of the aperture of the objective.

Having made these arrangements, take a pocket lens before the eye, and bend over the microscope until the Ramsden disc of the eye lens represented in Plate X, Figs. 1 and 2, comes clearly into view. There will be seen imaged in this Ramsden disc—as shown in Plate X, Fig. 3, A, B, and C, the equatorial plane of the objective vista, and in this again the equatorial plane of the condenser vista, which is positioned in the substage diaphragm.

Satisfy yourself that these things are so by widening and narrowing the substage diaphragm and verifying that its edge is imaged in accurate focus in the Ramsden disc of the eye lens which you are engaged in examining.

13. Configuration and course of the beams in the catena of the apertural planes.

It will suffice in this connexion to set forth the configuration and course of one of the component vistas of the catena here in question. The figure below shows the course of the rays in the vista which has its plane of origin in the apertural plane of the objective vista and its terminal plane in the Ramsden disc of the eye lens.

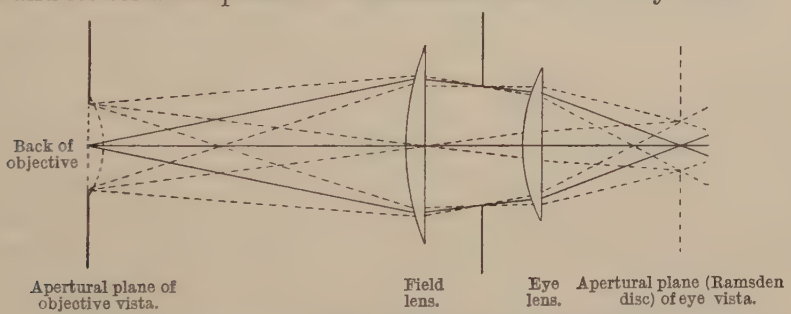


FIG. 65.

It will be seen that the beams which take origin in the radiant points which occupy the equatorial plane of the objective vista expand (under an angle which corresponds to the field angle of the objective) until they fall upon the field lens of the ocular. They are there converted into pencils of parallel rays.

These intersect, forming an apertural plane in the image plane of the objective vista in the diaphragm of the eyepiece.

Passing onwards, they fall upon the eye lens, and are by it converted into pencils of convergent rays. These come to focus, as already indicated, in the Ramsden disc of the eye-lens vista.

14. Scale of the image furnished in the Ramsden disc of the eye lens. On studying the diagram it will be manifest that the opening limb of the vista which is here in question is longer than the closing limb, and that the angular aperture of the closing limb is accordingly greater than the aperture of the opening limb. In conformity with this the image of the Ramsden disc of the objective, which occupies the Ramsden disc of the eye lens is, as appears in the figure, an image on a minified scale.

15. On the primary catena of the microscope considered as a whole, and on the terminal image which is furnished by its catena. Our study of the optical system of the microscope may be fittingly completed by considering the primary catena of the microscope as a whole.

Configuration of the catena of point vistas which lies upon the optical axis. This is set forth in Fig. 66 below.

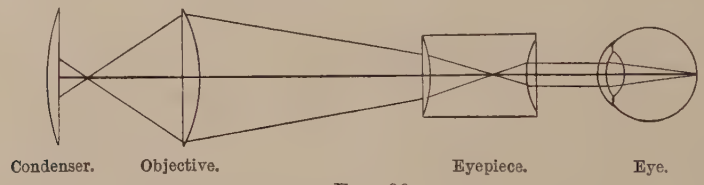


FIG. 66.

Inversion. We have seen that the image of the object upon the stage is inverted in the objective vista and re-inverted in the eye-lens vista. It is accordingly projected upon the retina in the form of an erect image, and is interpreted as an inverted image.

Scale of the image. The magnification of the terminal image is, as in the case of every catena of magnifying vistas, the product of the magnification achieved in the component vistas taken separately.

Procedures for measuring the cumulative magnification achieved in the primary catena of the microscope. The following represent a selection of the methods which are available for the measurement of the cumulative magnification achieved in the combined optical system of the microscope and eye.

(1) We can in two separate operations determine the magnification achieved in the objective vista separately and in the eye vista separately. We can arrive at the total magnification by multiplying these two factors.

(2) We can in two separate operations determine the magnifying power of the objective employed as a doublet to the eye and the magnification of the ocular regarded as an independent optical element, and we may then multiply together these two factors.

This method will be elucidated in the next chapter in dealing with the magnifying power of the separate optical elements of the microscope.

(3) We can place a micrometrical ruling on the stage of the microscope and read off the value of its divisions upon the scale of an eikonometer placed in position upon the eyepiece.

(4) We can, by means of a diffraction grating of appropriate periodical interval placed in front of the eye, effect the doubling of the lines of a micrometrical ruling placed upon the stage and seen by the aid of the microscope. Maintaining the periodical interval of the grating unaltered, we can in a second operation select from a series of progressively wider and wider interspaced rulings, placed at a distance of 250 mm. from the unaided eye, a ruling which is also doubled by the grating. The periodical interval of the ruling thus selected, divided by the periodical interval of the micrometrical ruling on the stage of the microscope, furnishes the magnification which has been effected by the microscope.

The principle of the method has been expounded in *Cap.* IX, *Appendix* II.

(5) We can determine the numerical aperture of the closing limb of the eye vista, and divide this into the numerical aperture of the opening limb of the objective vista.

The procedures which come into application in connexion with the three last enumerated methods of measuring the magnifying power of the microscope may here be briefly set forth and exemplified.

16. Procedure for measuring the magnifying power of the microscope by means of the eikonometer.

Place a micrometrical ruling upon the stage of the microscope and focus down upon it with the objective and ocular whose combined magnifying power is to be measured.

Having focussed its Ramsden eyepiece upon the micrometrical scale of the instrument, place the eikonometer upon the ocular of the microscope, arranging it so that the lines upon its scale may run parallel to those of the stage micrometer, and raising, or, as the case may be, depressing, the focussing lens in its sleeve in such a manner as to take in the whole field of the eye lens of the microscope.

Now read off the value of the divisions of the stage micrometer in terms of the divisions of the scale of the eikonometer, remembering that the divisions of the eikonometer scale have the value of millimetre divisions placed at the standard distance of 250 mm. from the eye.

Example. Four of the divisions of a stage micrometer which is ruled in hundredths of a millimetre are found to coincide with five of the divisions of the eikonometer scale.

Answer.—The magnification is 125-fold.

17. Procedure for measuring the magnifying power of the microscope by the aid of a stage micrometer and diffraction grating.

Place upon the stage of the microscope a ruling consisting of a succession of opaque and transparent lines and focus down upon it with the objective and ocular whose combined magnifying power is to be elicited. Take in hand a diffraction grating and dispose it in or near the apertural plane (Ramsden disc) of the eye lens of the microscope, with its rulings lying at right angles to the rulings of the stage micrometer. Looking down now into the microscope through the grating, rotate this last until the point is arrived at when the dark flanking lines, which in each case are derived from the dark object lines, fuse to make an intercostal line in the centre of the clear spaces, and when the bright flanking lines which derive from the bright object lines fuse in each case to make a bright intercostal line in the centre of the dark spaces.

Maintaining now the orientation of the grating, look through it at a system of lines—such as is to hand, *supra*, in Fig. 54, disposed at a distance of 250 mm. from the unaided eye, and take note of that pair of rulings which gives an intercostal line similar to that obtained in the first observation.

Now divide the periodical interval of the rulings which were seen as doubled under the microscope into the linear distance between the two lines which have in the second observation furnished the intercostal line.

The quotient represents the magnification effected by the microscope.

Example. A system of dark and bright lines, ruled $\frac{1}{176}$ mm. apart, is placed upon the stage of the microscope. A grating of similar periodical interval is interposed between the eye and the eye lens of the microscope. A clearly defined intercostal line appears in the centre of both the dark and bright bands, when the lines of the diffraction grating are disposed at an angle of 45° to the lines upon the stage.

Viewing with the unaided eye Fig. 54, from a distance of 250 mm., and holding the grating close up to the eye, with its rulings disposed at an angle

of 45° to the lines in the figure, it will be found that a median intercostal line is obtained in the case of the pair of lines which are placed 3 mm. apart.

Inasmuch as the microscope has here separated out lines placed $\frac{1}{16}$ mm. apart until they lie as far apart upon the observer's retina as lines 3 mm. apart seen without the microscope, the magnification achieved by the microscope is a 48-fold magnification.

18. Procedure for arriving at the magnification by dividing the numerical aperture of the opening limb of the objective and field-lens vista by the numerical aperture of the closing limb of the eye lens and eye vista.

The general principle upon which this method of determining the magnification of the microscope depends has been explained in *Cap.* VII, *subsect.* 35. We have seen there that whether we are dealing with a single vista or with a catena of vistas, the numerical aperture of the closing limb diminishes in each case *pari passu* with the magnification achieved. In conformity with this we can obtain the magnifying power of the microscope by dividing the numerical aperture of the opening limb of the objective vista by the numerical aperture of the closing limb of the eye vista.

Determination of the numerical aperture of the beam which enters the objective.

The determination of the numerical aperture of the beam which enters the objective involves (*a*) the measurement of the diameter of the back lens of the objective, or, in the case where the objective is not fully filled, the diameter of the illuminated area of the back surface of the objective, (*b*) the measurement of the focal length of the objective, and (*c*) a knowledge of the refractive index of the medium in which the object is mounted.

The first measurement (*a*) may be obtained where the back lens of the objective is fully filled, and where it is directly accessible to measurement by the application of a millimetre rule to the objective. In the case where the back lens is sunk, or where it is only partially filled by the transmitted beam, we obtain the measurement required by measuring the Ramsden disc of the eye-lens, and multiplying by the magnifying power of the ocular, as explained in *Cap.* XIII, *subsect.* 45.

The focal length of the objective (*b*) is arrived at most simply by measuring its magnifying power (*vide Cap.* XIII, *subsect.* 28), and dividing this magnifying power into 250 mm. or 10 inches.

From these measurements, and a knowledge of the refractive index of the medium in which the object is mounted, we obtain the numerical aperture of the beam which enters the objective in accordance with the formula—

$$\text{N.A.} = \frac{\text{semidiameter of beam} \times \text{refractive index of the immersion medium}}{\text{focal length of the objective}}$$

Determination of the numerical aperture of the beam which is furnished to the eye by the eye lens of the microscope.

The numerical aperture of the beam which is furnished to the eye by the eye-lens of the microscope is obtained by dividing the semi-diameter of the Ramsden disc of the eye lens (measured as explained in *Cap.* VII, *sec.* 39), by 250 mm. or 10 inches.

The magnifying power of the microscope is obtained from the numerical apertures of the opening and closing beams in accordance with the formula—

$$\text{Magnifying power of microscope} = \frac{\text{numerical aperture of beam which enters the objective}}{\text{numerical aperture of beam which enters the eye}}$$

Example 1. (*a*) The opening beam of the objective vista completely fills the back lens of the objective. The objective, which is a dry lens, has a focal length of 16 mm. The diameter of the back lens is 8 mm.

The numerical aperture of the opening beam is thus: $\frac{4}{16} = 0{\cdot}25$.

(*b*) The Ramsden disc of the eye lens measures 1 mm. The closing limb of the eye vista is taken as 250 mm.

The numerical aperture of the terminal beam is $\frac{0{\cdot}5}{250} = 0{\cdot}002$.

(*c*) Dividing the former of these expressions by the latter, we arrive at a magnification of 125.

Example 2. (*a*) The opening beam of the objective vista does not fill the back lens of the oil-immersion objective employed.

As a consequence, the diameter of the aperture of the objective vista cannot be arrived at by measuring the diameter of the back lens of the objective.

It can be arrived at by measuring the Ramsden disc of the eye lens and multiplying this measurement by the magnifying power of the ocular.

The Ramsden disc of the eye-lens measures 0·5 mm.

The ocular possesses a magnifying power of 4.

It follows that the aperture of the objective vista measures 2 mm.

The focal length of the objective is 2 mm.

The refractive index of the oil which bathes its front face is 1·5.

In correspondence with these data, the Numerical Aperture of the beam which enters the objective is $\frac{1 \times 1{\cdot}5}{2} = 0{\cdot}75$.

(*b*) The Numerical Aperture of the terminal beam is $\frac{0{\cdot}25}{250} = 0{\cdot}001$.

(*c*) The magnification $\frac{0{\cdot}75}{0{\cdot}001}$ is 750.

19. Quality of the terminal image, and on the superimposition upon this of an entoptic picture.

While the general consideration of the quality of the terminal image may be appropriately postponed until we come in *Cap.*

XIII to deal with the properties of the optical components of the microscope, and in *Cap.* XV to deal with the instrumental adjustments which are appropriate for the development of a critical image, we may here appropriately take cognizance of the fact that a projection picture of the pupillary aperture, or of a portion of that aperture, is in every case superimposed upon the image which is furnished by the microscope, and that this superimposed picture does when it acquires distinctness seriously obscure the microscopic image.

This will be clearly realized on carrying out the following experiment :—

Experiment. Having narrowed the substage diaphragm, and having fitted to the barrel of the microscope a high-power objective and a high-power ocular, focus down upon any brightly-illuminated microscopical preparation.

Now, using one eye only, view the Ramsden disc of the eye lens from a distance of some 100 mm.

When the Ramsden disc is viewed under these conditions, the image which is obtained is an unfocussed image. But it is, be it noted, quite other than the unfocussed image which would be obtained with an absolutely transparent lens disposed behind a clear-cut circular aperture. Instead of the absolutely sharp-cut and homogeneously illuminated disc of fixed dimensions which would in such case be obtained, we have here upon the retinæ a disc of coarsely granular texture, composed of dark and brightly scintillating points, delimited by a margin of spikes, and varying in size as the pupil of the observing eye dilates and contracts. There is, in fact, here in view a projection picture of the pupillary aperture which differs only with respect to its smaller dimensions and its coarser texture from the projection picture with which we made acquaintance in *Experiments* 1–3, *Cap.* X, *subsect.* 11, and in *Experiment* 2, *Cap.* XI, *subsect.* 9.

Now bring the eye nearer to the microscope, and note that, as the obliquer beams which come off from the Ramsden disc enter the pupil, and as the diffusion discs which correspond to the individual beams which take origin from radiant points in the Ramsden disc expand as these are brought nearer to the eye, the illuminated area on the retina grows larger and larger. The general appearance of the disc now corresponds to that which is represented in Fig. 4, Plate X.

Finally, bring the eye still nearer to the eye lens until the Ramsden disc comes to lie in, or a short distance in advance of, the pupillary aperture. Note that as the eye is brought into this position the granular disc continues to expand until its margin is hid behind the diaphragm of the ocular, and its texture opens out and acquires a certain transparency (Plate XVIII, B). Finally, take cognizance of the fact that under the conditions of the present experiment the blurring which is referable to the opacities of the ocular media never entirely disappears, and that the resultant pattern of light and shade hangs everywhere as a smoky haze over the microscopic image.

CHAPTER XIII.

ON THE OPTICAL ELEMENTS OF THE MICROSCOPE CONSIDERED SEPARATELY.

Mirror—The substage diaphragm—The substage condenser—Focussing of the substage condenser—Supplementary points in connexion with the focussing of the condenser—Centring adjustment of the substage condenser—Purpose of the centring adjustment of the condenser, and method of centring—Method of employing the condenser as an immersion system—Optical properties of the condenser—Focal length—Numerical aperture of the condenser—Measurement of the numerical aperture of the condenser—Method of obtaining from the condenser a wider beam than that which corresponds to its numerical aperture—Corrections of the condenser, and question of the relative advantages of the "Abbe" and "achromatic" condenser—Method of testing the corrections of the condenser—Slide and cover glass and media interposed between condenser and objective—Influence exerted upon the numerical aperture of the transmitted beam by the optical media interposed between condenser and objective—Distortion of the configuration of the closing limb of the condenser vista and the opening limb of the objective vista by the slide and cover glass and mounting medium—Distortion of the closing limb of the condenser vista by the interposition of the slide in the case where the microscopic object is mounted in air and the condenser is unimmersed—Distortion of the opening limb of the objective vista by the interposition of the cover-glass in the case where the microscopic object is mounted in air and the objective is unimmersed—The objective—Classification into dry and immersion objectives, and advantages of the latter in the case where highly magnifying systems are employed—Method of employing an oil-immersion objective—Focussing of the objective—Centring of the objective—Optical properties of the objective—Focal length and magnifying power of the objective—Method of measuring the focal length of the objective—On the numerical aperture of the objective and its influence upon the quality of the microscopic image—Measurement of the numerical aperture of the objective—Principle of the Abbe apertometer—Procedure for measuring the N.A. of the objective by means of the Abbe apertometer—Procedure for measuring the numerical aperture of the objective without recourse to the apertometer—On the accurate figuring and correction of the objective, and on the influence exerted by these factors upon the quality of the microscopic image—Corrections of the objective—Correction collar—Preliminary considerations in connexion with the testing of the accuracy of the figuring and the adequacy of the corrections—Procedure to be adopted in connexion with the proving of an objective—Consideration of the suitability of diatoms as test-objects for the proving of objectives—Employment of stained film preparations of bacteria (a) *as test-objects for the general accuracy of the*

figuring and corrections, (b) *as special test-objects for proving the chromatic corrections, and* (c) *as test-objects for proving the flatness of the image field—Objective changer—Barrel of the microscope and draw tube—Width of the barrel—The ocular—Determination of the magnifying power of the ocular—Corrections of the ocular.*

1. Mirror.

The mirror which is fitted to the microscope has ordinarily on one side a plane, on the other side a concave face.

The plane face furnishes, in the case where a distant light source such as the sky is employed, parallel rays (Fig. 67) ; in the case

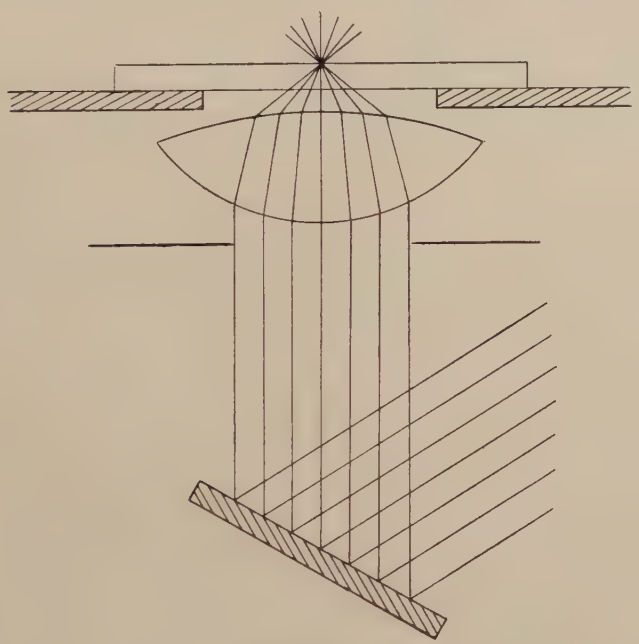


Fig. 67.

where a nearer light source, such as a lamp, is employed, divergent rays (Fig. 68).

The concave face of the mirror furnishes, in the former case, pencils of convergent rays (Fig. 69) ; in the latter case, pencils of more or less parallel rays (Fig. 70).

2. The substage diaphragm.

The light from the mirror is in every case transmitted to the microscope stage through a diaphragm in the substage, which can be opened up or narrowed as occasion may require. The *iris diaphragm* which can be seen in Plate XII, Fig. 1, furnishes the usual and most convenient arrangement for regulating the aperture.

By the introduction of suitable stops into the diaphragm, any portion of its aperture may be obtruded. The central spot stop (Fig. 71, A) serves for blocking out the centre ; the stop figured

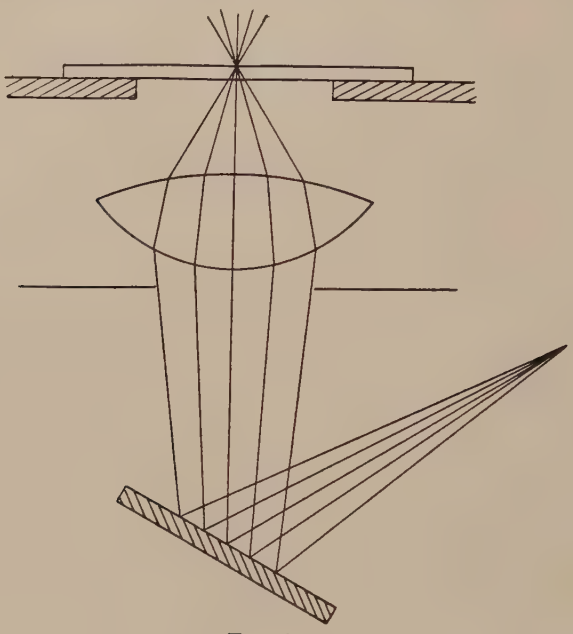


Fig. 68.

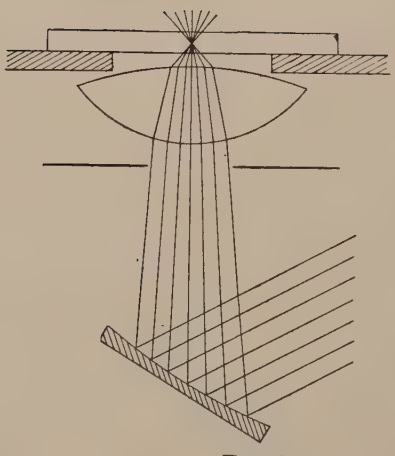


Fig. 69.

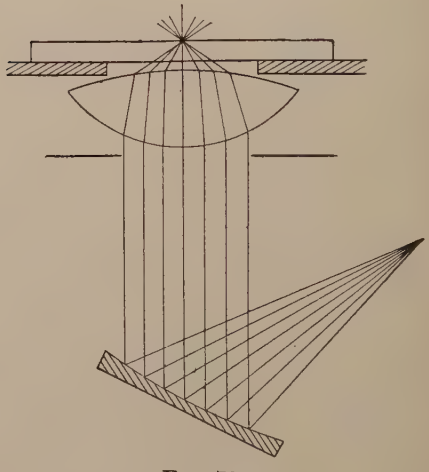


Fig. 70.

alongside of it (Fig. 71, B) for the blocking out of the centre and all but a small area of the peripheral zone of the aperture.

An effect similar to that obtained with the stop last mentioned

can be obtained (in the case of microscopes provided with a suitable mechanism in the substage) by closing down the iris diaphragm and bringing its aperture under the peripheral zone of the condenser (Plate XII, Fig. 1).

FIG. 71.

3. The substage condenser.

In the case of all but the very simplest microscopes, a condenser is fitted in the substage.

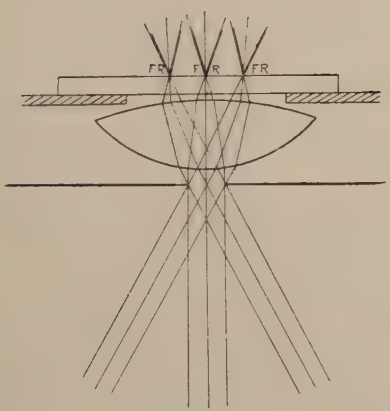


FIG. 72.

FR, FR, FR, FOCAL RADIANT POINTS FROM WHICH LIGHT RADIATES INTO THE OBJECTIVE.

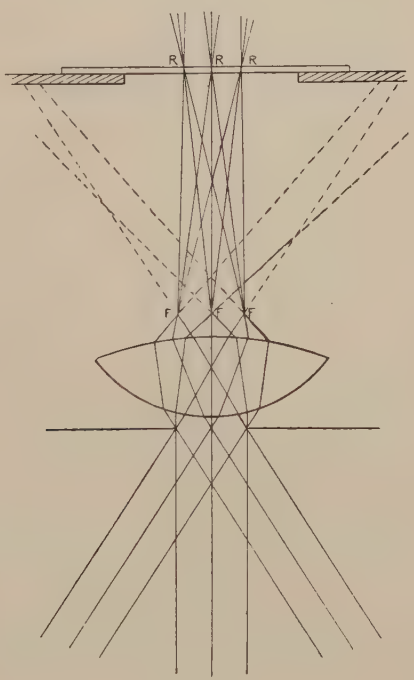


FIG. 73.

F, F, F, FOCAL POINTS; R, R, R, NODAL RADIANT POINTS, FROM WHICH LIGHT RADIATES INTO THE OBJECTIVE.

The condenser employed in association with suitable stops in the substage diaphragm allows of the illumination of the microscopic preparation by—

(1) A system of luminous points, each radiating light in the form of a solid cone along the optical axis of the microscope, and outwards in all directions within the compass of a small angle from that optical axis (Figs. 72 and 73).

(2) A system of luminous points, radiating light in the form of a solid cone along the optical axis of the microscope, and outwards in all directions within the compass of a considerable angle to the axis in question (Fig. 74).

(3) A system of luminous points, radiating light in each case in the form of a hollow cone outwards in all directions, in each case only at a considerable angle to the optical axis (Fig. 75).

(4) A system of luminous points, radiating light in the form of a narrow solid cone along an axis disposed obliquely to the optical axis of the microscope (Fig. 76).

(5) A system of luminous points, radiating light of one colour along the optical axis and outwards at a small angle from this, and light of another colour at a greater angle to the optical axis in the form of a hollow cone (Plate XI, Fig 1).

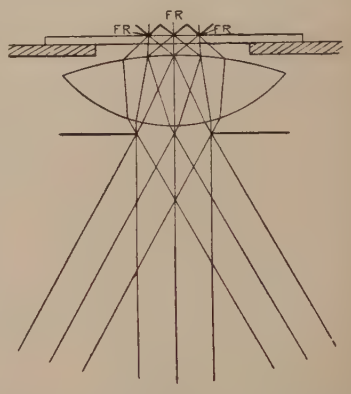


FIG. 74.

FR, FR, FR, FOCAL RADIANT POINTS FROM WHICH LIGHT RADIATES INTO THE OBJECTIVE.

(1) The first form of illumination is obtained, as will appear on consideration of the figures above, both when the transmitted beam is severely cut down by a stop in the substage (Fig. 72), and when the condenser is racked down in such a manner as to bring the beams to focus on a plane considerably below the stage of the microscope (Fig. 73).

(2) A system of luminous points, radiating light in the form of a solid cone along the axis of the microscope and outwards in all directions within the compass of a considerable angle to the axis in question is obtained when the substage diaphragm is fully open and the condenser has been focussed, as in Fig. 74, upon the plane upon which the microscopic object is disposed.

(3) The third form of illumination, known as *dark-ground illumination* (Fig. 75), is obtained when, after making the

arrangements described in (2), the central portion of the condenser is afterwards blocked out by a central spot stop (Fig. 71, A).

(4) The fourth form of illumination known as *oblique illumination* (Fig. 76) is obtained when the condenser has been focussed as in (2), and when, further, all but a small area of the outer zone of the condenser has been obtruded by a stop (Figs. 71, B).

(5) The fifth form of illumination is obtained when the condenser has been focussed and when a parti-coloured stop has been placed in the substage (Plate XI Fig. 1).

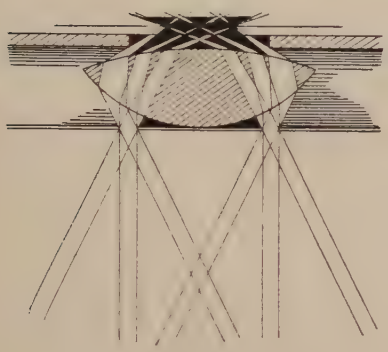


Fig. 75.

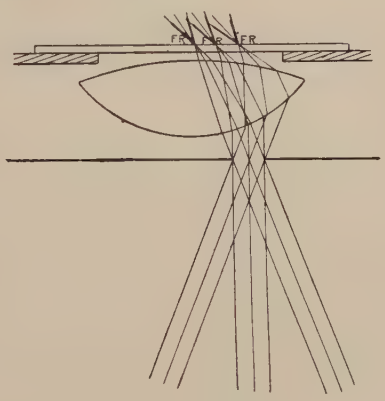


Fig. 76.

4. Focussing of the substage condenser.

It will have been realized that for every type of illumination, except the first mentioned, the rays from the periphery of the condenser must strike upon the microscopic object. To achieve this, the condenser must be focussed (or approximately focussed) upon the optical plane upon which the microscopic preparation is disposed.

To this end the substage, which carries the condenser, is fitted with a focussing movement.

The focussing of the condenser may be effected by two different procedures. Each of these involves three separate operations :—

(1) *Ordinary procedure.*

(1) In a first operation the optical plane upon which the condenser is to be focussed is brought into view under a low-power objective.

(2) Provision is then made for the ready identification of the terminal plane of the condenser vista. This is done by directing the mirror to any light source which will give a

sharply characterized image or, failing such distinctive light source, by bringing into the field of the mirror some unmistakable and moderately distant object—such as the cross-bar of the window-frame, as in Plate II, *a*, and Plate III, *d*, or, as in the case of Figs. 77 and 78, a system of squares—which will supply in the terminal focal plane of the condenser vista a characteristic feature such as is required for its ready identification.

(3) Lastly, the condenser is racked up or down until the image of the light source, or of the selected intrusive object, is brought into the optical plane upon which the microscopic object is disposed.

The details of the procedure are as follow :—

Place upon the stage of the microscope the microscopic preparation upon which the condenser is to be focussed. Fit a low-power objective and a low ocular to the barrel of the microscope, and focus down upon the preparation.

In the case where daylight is employed, use the plane face of the mirror and bring into its field either the window bar or, as the case may be, clouds or a distant landscape.

In the case where a lamp furnishes the light, bring the flame into the field of the concave mirror.

Narrow the substage diaphragm in such a manner as to cut down severely the beams which are transmitted through the condenser, and to obtain in this manner both a sharper image and an image extending through a greater depth of focus.

Keeping the eye at the ocular of the microscope and transferring the hand from the focussing adjustment of the microscope to the focussing adjustment of the condenser, rack this last up or down until a sharp image of the external object (lamp flame, window bar, clouds, or, as the case may be, distant landscape) is seen projected upon the microscopical preparation.

The condenser is now in focus.

If now a still finer adjustment of the focus is required, open wide the substage diaphragm so as to obtain the finer resolution in depth and the shallower image, which is, as we have seen, furnished by a wider aperture (*Cap.* VII, *subsect.* 28), and now refocus.

Figs. 77 and 78, which are here subjoined, furnish illustration of the optical conditions which obtain respectively in the case where the condenser is focussed on an optical plane lying below the stage of the microscope and on the optical plane which carries the microscopic object.

The microscopic objects on the stage are here in the principal figure represented by S, S, S, and in the inset figures (corresponding to the magnified images seen in the microscope) by circular contours. In the field of the mirror there are supposed to lie in each case rulings forming a system of squares. These rulings are represented in the princi-

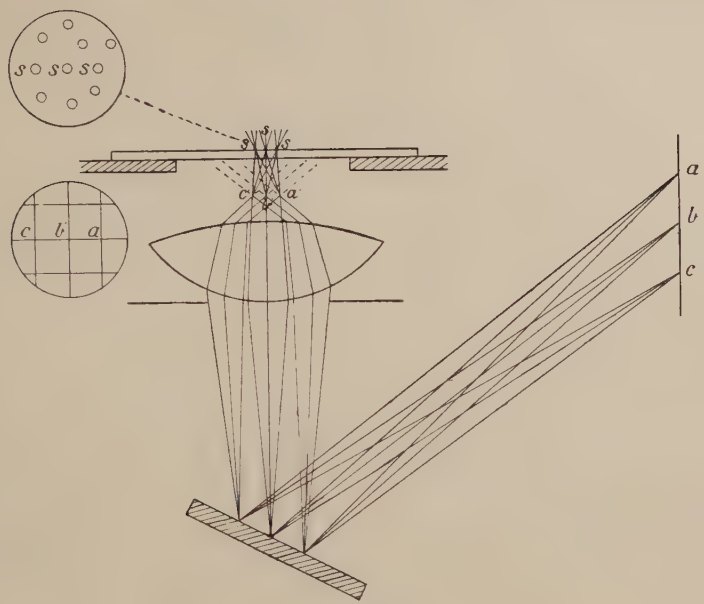


FIG. 77.

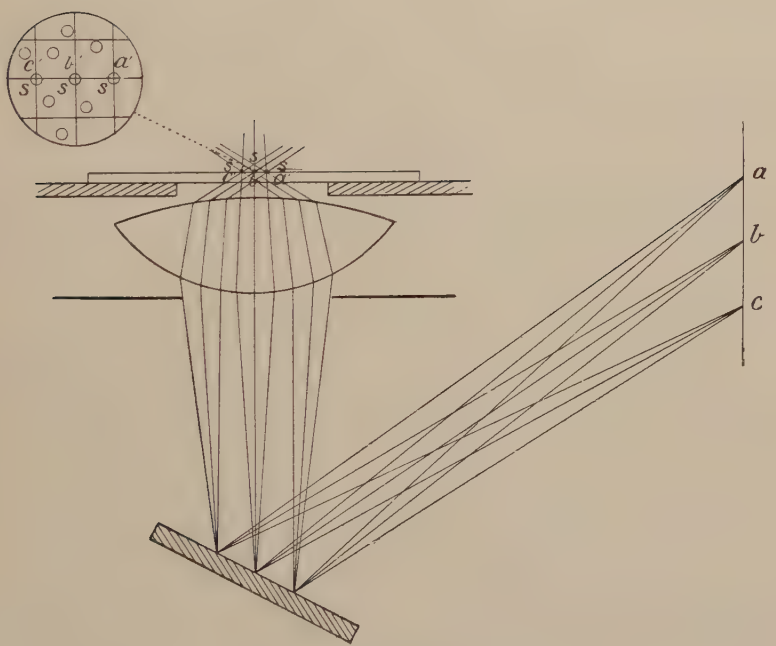


FIG. 78.

pal figures by the points a, b, c; and their images by a', b', c'. In the inset figures we have in each case a minified image of the squares.

In Fig. 77, where the condenser is represented as racked down, the terminal plane of the condenser vista, which carries the image of the system of squares, is positioned considerably below the level of the microscopic preparation upon which the objective would be focussed. In accordance with this, the terminal plane of the condenser vista, which carries the image of the squares, and the plane of origin of the objective vista, which carries the microscopic object, are in the figure depicted on two separate inset figures.

In Fig. 78, where the condenser is racked up in such a manner as to concatenate the condenser vista with the objective vista, the terminal plane of the former vista has coalesced with the plane of origin of the latter vista. In accordance with this, the microscopic preparation and the figure of squares, which has been superposed upon the preparation by the focussing of the condenser, are conjointly depicted on a single inset figure.

(2) *Alternative procedure.*

This again involves three separate operations :—

(1) In the first, as in the ordinary procedure, the microscopic object upon which the condenser is to be focussed is brought into view under a low-power objective.

Here the microscopic object must consist of highly refractive elements, mounted in a medium of low refractive index. A filament made by drawing out a piece of glass in the flame, or, better, a floccule of glass wool, furnishes an ideal object for the purpose when mounted in air or water.

(2) In a second operation, provision is made for the identification of the rays which come into focus from the peripheral zone of the condenser. The identification of these rays can be effected either (*a*) by obtruding, by a central spot stop, all the central area of the condenser while leaving the peripheral zone of the condenser unobtruded ; or (*b*) by employing in the substage a parti-coloured stop, which transmits light of one particular colour to the central part of the condenser, and light of another colour to the peripheral part of the condenser.

(3) In a third operation the condenser is racked up and down until the highly refractive objects are seen outlined by bright outlines on a dark field or, as the case may be, by light of one colour on a field of another colour.

Experiment 1. Place upon the stage of the microscope a blank slide and focus down upon it its upper surface with a low-power objective, and focus the condenser upon this optical plane.

PLATE XI.

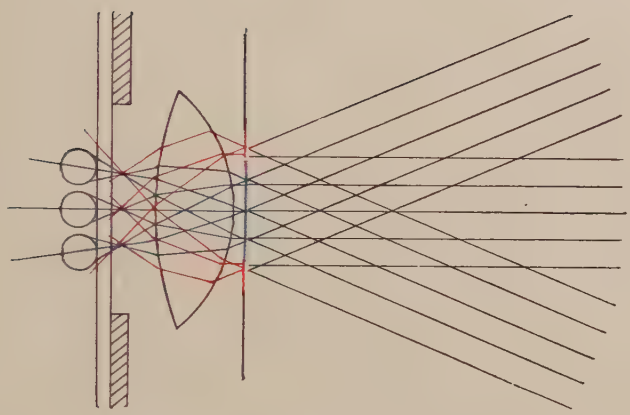


FIG. 2.

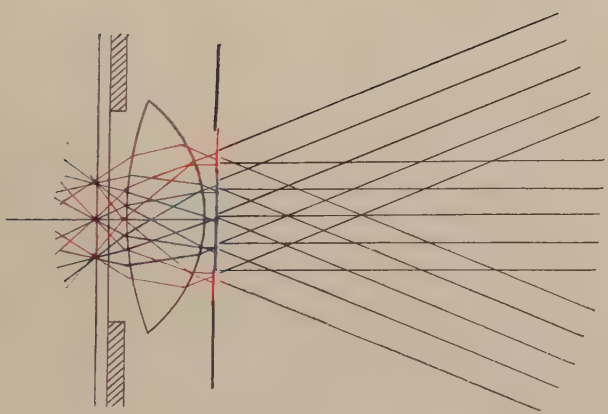


FIG. 1.

Now throw open the substage diaphragm to its fullest extent, and introduce into it a central spot stop of such dimensions as to cut out from the centre of the wide cone of light which comes off from the radiant points on the microscope stage all those rays which before entered the aperture of the objective.

In conformity with the fact that the whole of the light which before radiated from the stage into the aperture of the objective is now cut off, the field of the microscope will now be dark.

Now place upon the surface of the slide some filaments of glass wool, mounting these in water under a cover glass. The filaments will now, as explained in *Cap.* II, refract and reflect the light which impinges upon them, directing it upwards in such a manner as to enter the aperture of the objective.

In consequence of this the filaments will now appear in the field of the microscope outlined by bright outlines upon a dark field (cf. Fig. 75, with Fig. 5).

Now rack down the condenser so as to bring down its image plane below the level of the microscope stage. In association with this the bright outlines will be extinguished.

A consideration of Fig. 73 will show that the oblique rays which derive from the focal points upon the terminal plane of the condenser vista will now go wide and will no longer strike upon the glass filaments.

Experiment 2. Substitute for the central spot stop a parti-coloured stop, such as is shown in Plate I, Fig. E, 3, and go through exactly the same procedure.

Note that (*a*) where the condenser is in focus and the glass filaments are on the stage of the microscope, these are outlined with red upon a field of blue (Plate II, m), (*b*), where the condenser is in focus and the object is removed, the field is of a uniform blue, (*c*) where the condenser is thrown out of focus and the filaments are on the field, these last are delineated by dark outlines on a field of blue (Plate II, *k*).

A comparison of Plate XI, Fig. 1, with Fig. 2 on the same plate, will make clear that the extinction of the red outlines is associated with the fact that the red rays which here come off at a considerable angle to the optical axis fail in the case where the condenser is racked down to impinge upon the glass filaments.

NEW YORK PUBLIC L

5. Supplementary points in connexion with the focussing of the condenser.

The following supplementary points in connexion with the focussing of the condenser deserve attention :—

(1) When once the condenser has been focussed, the external object—let us say, the window bar—which has subserved the purposes of focussing, may with advantage be thrown out of the field of view by rotating the mirror upon its axis. In the case where the microscopic preparation is to be examined under a high power, it will suffice if the image of the external object

be moved out of the centre of the field of the lower power objective.

(2) If through inadvertence the concave, in lieu of the plane face of the mirror, has been employed in the focussing procedure described above, it will in most cases be impossible to bring the light from a distant light-source to focus on the microscopic preparation. In such a case—as is shown in Fig. 69—the beams will have been rendered convergent by the mirror before they fall upon the condenser, and will, in consequence, come to focus on a plane below that upon which the microscopic object is disposed.

(3) Owing to the short working distance of the substage condenser, similar difficulties will be experienced even with the plane mirror in the case where the microscopic preparation is mounted on too thick a slide.

(4) Owing to the circumstance that the focussing of the condenser is in reality an adjustment of the height of the condenser to the thickness of the slide upon which the particular microscopic preparation is mounted, it will be necessary to focus the condenser anew every time that a preparation mounted on a thicker or thinner slide is substituted for the one already under the microscope. It follows from the same consideration that the substitution of a high for a low objective, or vice versâ, does not entail re-focussing of the condenser.

Experiment 1. Placing any ordinary microscopic preparation on the stage of the microscope, carry out the ordinary procedure for focussing the condenser as described above, varying it only by employing the concave in lieu of the plane mirror. It will be found that in the case where the microscopic preparation is disposed on a slide of ordinary thickness the image of the distant light source, or external object which has subserved the focussing operation, will, even in the case where the upper face of the condenser is in contact with the lower surface of the microscopic slide, be formed below the plane upon which the object is disposed. It will generally be formed within the glass slide.

Experiment 2. Having verified that the difficulty which interferes with the focussing of the condenser disappears on reverting to the plane mirror, interpose now a slide of ordinary thickness between the upper face of the condenser and the slide which carries the microscopical preparation. Note that it is no longer possible to bring the condenser to focus on the microscopic object. This difficulty arises in actual practice in the case where the microscopic object is mounted on too thick a glass slide.

Experiment 3. Focussing the condenser on any ordinary microscopical preparation and keeping the image of the external object which

has subserved the focussing operations in the centre of the field, replace the lower-power objective which has been employed in the focussing operations by a high-power objective. Note, on focussing down with this on the preparation, that the image of the external object still continues in view. Now reverting to the lower-power objective, substitute or replace the first microscopic preparation by another mounted on a slide of different thickness. On adjusting the focus of the microscope so as to bring this new microscopic preparation into view, it will be found that the image of the external object has been lost sight of.

6. Centring adjustment of the substage condenser.

In the higher class microscopes—more particularly in those of English make—the substage condenser is fitted with centring screws, which allow of the condenser being properly centred. In continental microscopes a substitute for the centring of the condenser is provided in the form of a centring arrangement attached to the substage diaphragm.

7. Purpose of the centring adjustment of the condenser, and method of centring.

We shall see in *Cap.* XV, *subsect.* 2, that, whenever beams traversing the aperture of a lens obliquely are cut down by the edges of that aperture, the effect upon the picture is exactly the same as where the beams pass through an aperture which has been stopped down in an unsymmetrical manner. We obtain in each case an unsymmetrical antipoint which may interfere with resolution. This unsymmetrical cutting down of the beams in the aperture will obviously be at a minimum where the axis of collimation of the condenser is brought into line with the axis of collimation of the objective. This is the operation which is technically known as the centring of the condenser.

The axis of collimation of the condenser will be in line with the axis of collimation of the microscope when a narrow beam, sent through the centre of the condenser, passes up through the centre of the objective, to emerge through the centre of the upper end of the barrel of the microscope. The actual operation of centring involves the following steps : (*a*) The closing down of the substage diaphragm till it forms a pin-point aperture directly under the centre of the condenser. (*b*) The projection of a beam of light through the pin-point opening in the substage diaphragm and through the centre of the substage condenser. (*c*) The displacement of the condenser in the horizontal plane (by centring screws or otherwise) until the position is found at which the beam which traverses the centre of the condenser traverses the centre of the

aperture of the objective. (*d*) The fitting into the barrel of the microscope tube of a "centring cap," i.e. a cap provided with a pin-hole opening, which falls in the exact centre of the microscope tube. (*e*) The further displacement of the condenser until the point is found at which the beam which traverses the centre of the condenser and the centre of the objective is transmitted through the aperture in the centring cap to the observer's eye.

Procedure. Fit to the microscope tube the objective you propose to use, stop down the substage diaphragm to its narrowest limits, and project the light from the window by means of the mirror upon the lower face of the condenser.

Bring into view the back of the objective, either by examining through a pocket lens the Ramsden disc of the eye lens, or by removing the ocular and looking down directly on to the back of the objective. If now the condenser is in allineation with the objective, the beam which emerges from the back of the objective will be positioned as in Plate X, Fig. 3, B. In the case where the condenser is out of centre, the appearances will be as in Plate X, Fig. 3, C.

In this latter case adjust the position of the condenser by means of the centring screws—or, in the case of the continental type of microscope, adjust the position of the substage diaphragm by means of its centring adjustment—until the beam which emerges from the back of the objective is transferred from the excentric position which it occupies in Plate X, Fig. 3, C, to the central position which it occupies in Plate X, Fig. 3, B.

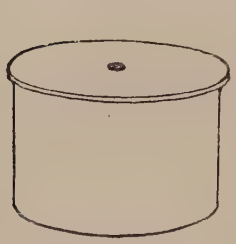


Fig. 79.

Now—if this has not been previously done—remove the ocular, and substitute for it the centring cap (Fig. 79).

Having placed the eye behind the pin-point aperture in this cap further readjust the position of the condenser by means of the centring screws until the beam of light is transmitted to the eye.

8. Method of employing the condenser as an immersion system.

In the case where we desire to fill in the aperture of an oil immersion objective by a beam of large N.A., and in the case where we desire to project upon the specimen on the stage of the microscope very oblique rays, the condenser must be employed as an immersion system.

The condenser is immersed by placing a large drop of cedar oil (or, as the case may be, of water) upon its upper face, another similar drop upon the lower surface of the slide, and then bringing the two drops in contact by replacing the slide upon the microscopical stage and racking up the condenser until the intervening

Plate XII.

Fig. 1.

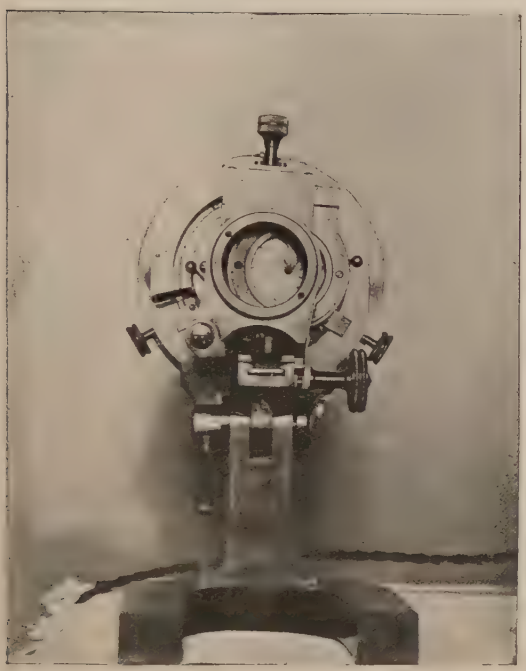


Fig. 2.

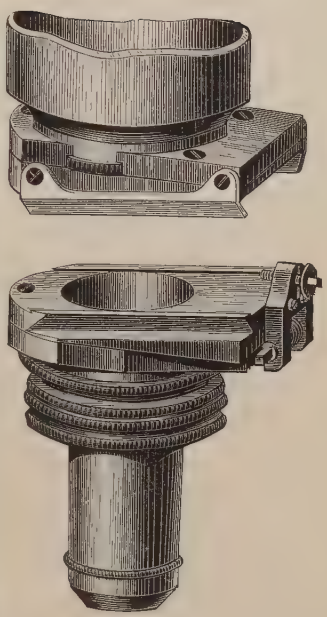
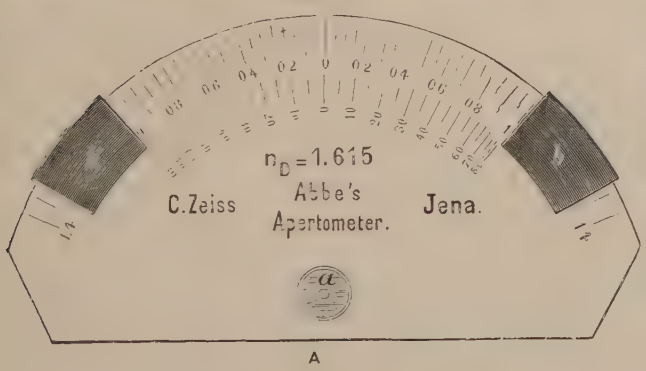


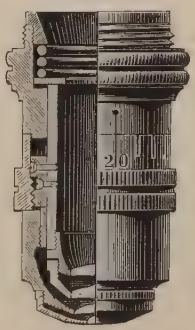
Fig. 3.



A

B

Fig. 4.



layer of air is expelled by the running together of the drops of oil.

In the case where it is desired to immerse the condenser without disturbing the object under the microscope, the cedar oil can be carried down by means of a capillary pipette through the aperture in the stage to the upper face of the condenser.

9. Optical properties of the condenser.

We have to consider under this heading the focal length of the condenser ; further, its numerical aperture and its corrections.

10. Focal length.

The focal length of the condenser is chosen by the maker in such a manner as to bring the light to focus under the largest possible angle upon an object disposed upon a slide of ordinary thickness. In actual practice a focal length of 8 to 14 mm.—giving a working distance of 2 to 3 mm. for the thickness of the slide—is adopted.

Measurement of the focal length of the condenser.—Where it is desired to determine the focal length of the condenser, this may conveniently be deduced from the scale of the image.

We may, on the one hand, place an object of known size in a distant window and measure by means of a rule the distance between microscope and window, and by means of a stage micrometer the dimensions of the minified image of the object which is furnished by the condenser. The minification of the image will in such a case correspond to the ratio which the interval between window and microscope bears to the focal length of the condenser.

Or, alternatively, we may measure by means of the eikonometer the scale of the image which is furnished by the condenser when working at its principal focal distance. The procedure would be as follows :—

Procedure. Dismount the condenser, invert it, and look through it at a millimetre scale, increasing the distance between the condenser and scale until the point is arrived at where this can no longer be done without prejudice to the image. Now place the eikonometer on the surface of the condenser, and read off the number of divisions of its scale covered by the image of each millimeter division of the rule. The number so arrived at, divided into 250 mm., gives the focal length of the condenser.

11. Numerical aperture of the condenser.

The numerical aperture of the condenser corresponds to the numerical aperture of the beam which emerges from the condenser in the case where the substage diaphragm is fully open, and where no optical obstacle prevents the emergence of the complete beam

from the upper face of the condenser. The optical obstacle which comes into consideration here is that constituted by a stratum of air in contact with the upper face of the condenser. Where such an air stratum overlies the condenser, every ray which impinges upon that stratum at an angle of 42° is deflected so as to run parallel to the surface of the condenser, and every ray which impinges at an angle greater than this (*the critical angle*) is turned back by total reflection.

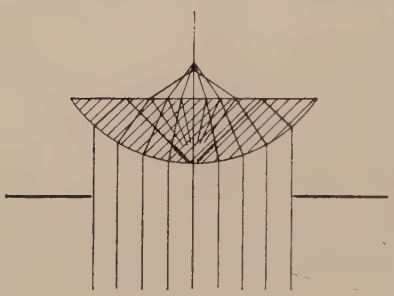


Fig. 80.

In accordance with the fact that the critical angle is reached as soon as the divergence angle of the emergent beam reaches 90° and its full angular aperture reaches 180°, the numerical aperture of a beam delivered through air cannot exceed 1.

Beams of larger numerical aperture are delivered from the condenser only in the case where an immersion fluid is employed.

Experiment. Employing a condenser whose n.a. (ascertained as in *subsect.* 12, *infra*), exceeds 1, focus it upon any convenient microscopic preparation.

Then substitute for the low-power objective an oil-immersion objective possessing a N.A. equivalent to that of the condenser, and focus down with this upon the specimen.

Bring into view now the back of the objective by scrutinizing the Ramsden disc of the eye lens by the aid of a pocket lens. It will be seen that the back of the objective is only partially filled in with the beams which proceed from the microscope stage. Now rack down the condenser and place, by the aid of a capillary pipette carried down through the aperture in the microscope stage, a drop of cedar oil upon the upper face of the condenser. This done, rack up the condenser again into place, and verify by scrutiny of the Ramsden disc of the eye lens that the back of the objective is now fully filled in or more fully filled in. This is the result of the delivery from the condenser of a beam of larger numerical aperture.

12. Measurement of the numerical aperture of the condenser.

The most convenient method of measuring the n.a. of the condenser is a method which is based upon the consideration that, in the case of two-lens systems which are furnishing a catena of vistas, the numerical aperture of the opening angle of the succeeding vista, in the case where the beam is passing from the one-lens system into the other without mutilation, corresponds exactly with the numerical aperture of the closing limb of the preceding vista.

Procedure. Placing any convenient microscopic object upon the stage, focus the condenser upon the preparation and open the substage diaphragm to its fullest extent, and eliminate the obstructing stratum of air by filling in with cedar oil the interspace between the upper face of the condenser and the lower face of the slide.

Then fit to the microscope tube an oil-immersion objective which possesses a very high numerical aperture and focus down with this upon the image plane of the condenser.

Examine now with a pocket lens the image of the back of the objective which is furnished at the Ramsden disc of the eye lens, and determine the dimensions of the illuminated area upon a scale disposed in the optical plane of the Ramsden disc.

Multiply this measurement by a number corresponding to the magnifying power of the ocular (*vide infra, subsec.* 45), and having obtained in this way the dimensions of the Ramsden disc of the objective, divide by two to obtain its semi-diameter and again by the focal length of the objective. The numerical aperture of the opening limb of the objective vista which is thus arrived at corresponds to the numerical aperture of the condenser.

Example. A convenient example of the working of the method is obtained by taking the hypothetical case which is presented in Fig. 81. We have here a case where the condenser vista is concatenated with the objective vista, and where the beam from the condenser passes without mutilation into the objective, completely filling in its aperture.

The semi-diameter of the objective in the figure will be found to measure approximately 12 mm.; and the focal length of the objective (measured between the pole of origin of the beam on the microscope stage and the edge of the lens) 15 mm. The medium in which that focal length is measured is oil possessing a refractive index of 1·5.

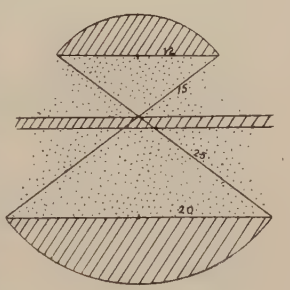


FIG. 81.

The actual dimensions of the figure as here reproduced will be found to be in each case a little smaller than those set down in the text.

In accordance with these data, the N.A. of the beam which enters the objective would be $\frac{12 \times 1{\cdot}5}{15} = 1{\cdot}2$.

The n.a. of the condenser will accordingly be 1·2 also.

Verification. The semi-diameter of the condenser in the figure measures 20 mm.; the focal length is 25 mm. The refractive index of the medium in which the focal length is measured is 1·5.

$$\frac{20 \times 1{\cdot}5}{25} = 1{\cdot}2$$

13. Method of obtaining from the Abbe condenser a wider beam than that which corresponds to its numerical aperture.

We have seen that the n.a. of the condenser corresponds to

the n.a. of the beam which it brings to focus in the case where all obstructions have been removed.

We have now to realize that in the case where we have an extended light source, there may be found, upon an optical plane lying below the optical plane upon which the light source is brought to focus by the condenser, a system of radiant points, which receive

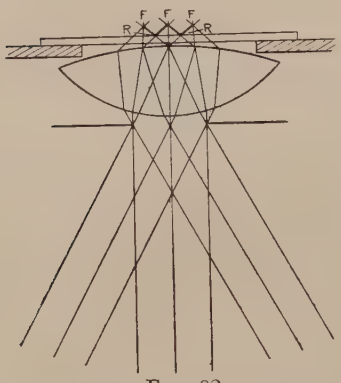


FIG. 82.

F, F, F, FOCAL POINTS POSITIONED ABOVE THE OPTICAL PLANE UPON WHICH THE OBJECTIVE IS FOCUSSED; R,R, NODAL RADIANT POINTS FROM WHICH LIGHT RADIATES INTO THE OBJECTIVE.

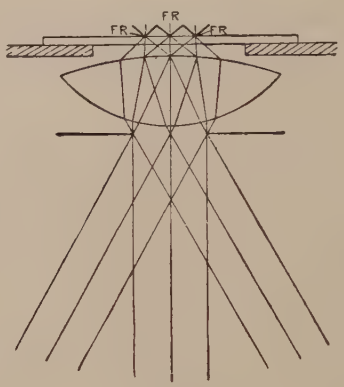


FIG. 83.

FR, FR, FR, FOCAL RADIANT POINTS FROM WHICH LIGHT RADIATES INTO THE OBJECTIVE.

and emit light under a larger angle than the actual focal points of the system.

The radiant points here referred to are in Fig. 82 above denoted by the letters R, R, the focal points being denoted by the letters F, F, F. The figure, which may with advantage be compared with Fig. 83, makes plain to the eye that, where the condenser is racked up so as to give a focussed image of an extended light source upon a plane superficial to that occupied by the microscope preparation, we have disposed in the optical plane occupied by this last a system of radiant points which receive and emit in each case a beam of larger numerical aperture than that of the condenser.

Experiment. Fit to the microscope tube a low-power ocular and an objective of moderate focal length, such as a one-third or one-fourth inch, and focus upon the upper surface of a blank microscopic slide.

Employing an extended light source, now focus the condenser upon this optical plane. Then bring into view the posterior surface of the objective and cut down the beam by shutting down the substage diaphragm.

Now rack the condenser up in such a manner as to bring the image of the light source into an optical plane superficial to the plane upon which the objective is focussed.

Note, as you rack up the condenser beyond its focus, that the diameter of the illuminated area at the back of the objective expands.

14. Corrections of the condenser, and question of the relative advantages of the "Abbe" and "achromatic" condenser.

Two types of condenser are fitted to microscopes.

In the one, width of numerical aperture is sought at the expense of accurate correction. In the other, the optician makes it an object of endeavour to furnish the largest possible aplanatic cone.

The well-known *Abbe condenser* conforms to the former type; the *achromatic condensers*, which are employed by many English microscopists, to the latter type. These English workers have hotly contended that the more expensive achromatic condenser possesses many advantages over the Abbe condenser. To the present writer the question presents itself in the following light :—

(*a*) The function of the condenser is not that of furnishing a perfectly corrected image of the light source, but rather that of furnishing on the stage of the microscope such radiant points as may be required for the development of the appropriate stage picture and for the proper representation of the stage picture in the microscopic image.

(*b*) Provided that the system of radiant points which is required for the development of each type of stage-picture and for the proper representation of the picture in the microscopic image is duly furnished, it is a matter of indifference whether it is placed at the disposal of the microscopist in the focal points of a corrected condenser or in the nodal points formed by the intersection of rays derived from different points in the light source.

If the contentions (*a*) and (*b*) are accepted, and if it can be shown that we have at disposal in the nodal points which are furnished by the Abbe condenser luminous points which radiate light indistinguishable in colour from that of the original light source under an angle which is as great or greater than that at which light is emitted from the focal points of the achromatic condenser, then the uncorrected condenser will be justified. Again, the Abbe condenser will be justified if it can be shown that under the conditions of ordinary microscopic work the system of radiant points which is furnished upon the stage by the achromatic condenser will not be very appreciably different from those furnished by the Abbe condenser.

Both the above points can be established :—

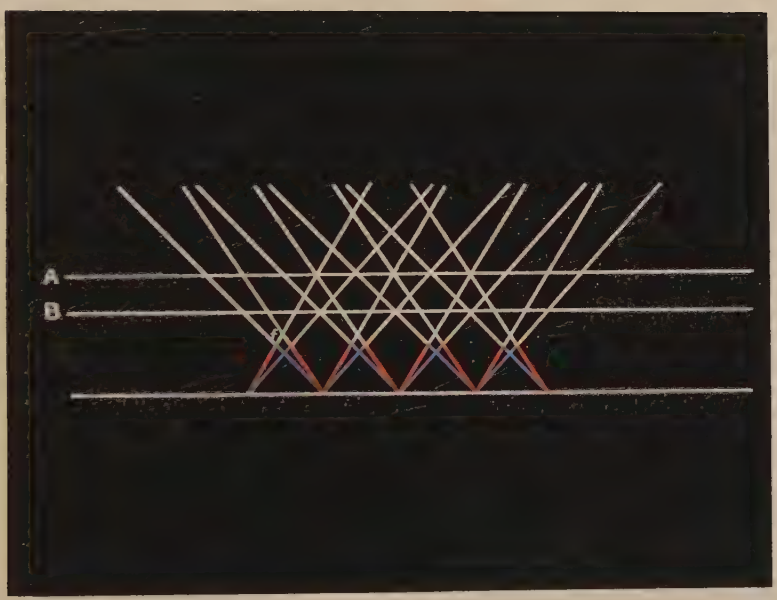
In connexion with the first the reader will appreciate on turning to Plate XIII that where the blue and red rays of an uncorrected lens system cross we have developed in each case a radiant point which emits light of the same character as that furnished by the original radiant source. This automatic colour correction is in point of fact very complete, and fails only where, as in *Experiment* 1 below, a bar of shadow lies across a field. As in the case of the chromatic aberration, so also in the matter of the cutting down of the beam which results from the uncorrected spherical aberration, automatic correction is obtained in the Abbe condenser. We have to take into consideration here not the case of an isolated beam coming to focus, as in Fig. 38, A, in the form of a caustic, and furnishing at the focal point of the central rays a beam of diminished n.a.; but have instead to consider the case where a number of beams derived from an extended light source are brought to focus side by side. Here the narrow beams derived from the more remote focal points, (*vide* Fig. 38, A) will be supplemented and widened out in each case by oblique rays from the foci of the delimiting rays of the adjoining caustics. And as a result there will be furnished by the incompletely corrected condenser upon the stage of the microscope luminous points which will radiate light under an angle not less than that under which light would be radiated by the focal points of an achromatic system of the same numerical aperture.

In connection with the suggestion made above that in the case where an achromatic aplanatic condenser is fitted to a microscope, the conditions of ordinary microscopic work may very often be such as to interfere with the development of properly corrected *foco-radial* points in the microscopic preparation, the following must be taken into consideration :—

(1) While a condenser which is designed to give a correct image in air cannot give a corrected image in oil, or vice versâ, it is almost essential that one and the same condenser should, in the course of microscopic work, be employed, as occasion arises, at one time as a dry system and at another time as an immersion system.

(2) The corrections of a condenser which is constructed so as to give a corrected image in air will, as will appear below, be thrown out of adjustment when a highly refracting element such as a microscope slide is interposed between the condenser and its image plane.

Plate XIII.



15. Method of testing the corrections of the condenser.

In testing the corrections of the condenser, we must take into consideration the circumstance that the corrections of any lens system are thrown out where the beams pass to their focus through variable thicknesses of media of different refractive index, and, in particular, that the corrections of the condenser will, as shown *subsect.* 18, *infra*), be thrown out when the beams pass, as they do in the ordinary case, first through air and then through a variable thickness of glass slide.

The following experiments are designed so as to avoid, so far as possible, these sources of error in testing the corrections of the condenser :—

Experiment 1. Throwing open the substage diaphragm to its fullest extent, rack up the condenser until the image of the window bar or other suitable external object is brought to focus on the microscope stage, or somewhere in the neighbourhood of it. Focus down upon this image with a low-power objective. Note that coloured fringes are developed along the edges of the bar in the case where the corrections of the condenser are imperfect. (Plate II, *a*, and Plate III, *d*.)

Close down the diaphragm and note the improvement which is achieved in the image.

Experiment 2. Place a sheet of blue glass under the fully open substage diaphragm and focus the image of the window as in the last experiment upon the plane of the microscope stage. Bring this image into view under a low-power objective.

This done, withdraw the blue glass and substitute for it a sheet of ruby glass.

Note that the image of the window bar in the case where an uncorrected condenser is employed practically disappears from view. Note also that it can be recovered on racking down the condenser or, alternatively, on racking back the barrel of the microscope.

Close down the substage diaphragm and note that the substitution of the red- for the blue-light filter, or vice versâ, has a much less conspicuous effect.

Experiment 3. Inscribe with a glass-ruling pencil a system of ruled squares on the window pane, focus the image of squares upon the optical plane of the microscope stage, and bring it into view under a low-power objective. Where an uncorrected condenser is employed it will be found that the diameter of the central squares exceeds the diameter of the squares which occupy the more outlying portions of the field.

Experiment 4. Employing the illuminating apparatus described in *Cap.* XV project upon the stage of the microscope a focussed picture of the restricted aperture which functions as the source of light, and focus down upon it with a low-power objective. Note that in the case where the condenser is uncorrected the bright image of the illuminating

aperture is surrounded with a wider, less brilliantly illuminated area, which fades out when the substage diaphragm is shut down. This encircling annulus of light represents the out-of-focus picture which derives from the peripheral parts of the condenser.

16. Slide and cover glass and media interposed between condenser and objective.

The slide, cover glass and the media which are interposed between the condenser and objective have to be considered from a twofold point of view. They may influence the microscopic image in particular :—

(*a*) By limiting the numerical aperture of the transmitted beam ; and

(*b*) By altering the configuration of the beam in the condenser and objective vistas respectively, and thus affecting the corrections.

17. Influence exerted upon the numerical aperture of the transmitted beam by the optical media, interposed between condenser and objective.

It will conduce to the simplification of the following discussion if we here leave out of consideration the microscopic object, and assume that we have in each case, upon the stage of the microscope, only a slide and cover glass, and between these a medium like Canada balsam, possessing a refractive index equal to that of glass.

Four cases present themselves for consideration :—

(1) In the first, illustrated in Fig. 84, the condenser and objective are both working through air, and are both focussed upon the upper surface of the slide. Here the light, which is on passage from condenser to objective, impinges in succession upon four refracting and reflecting surfaces.

It impinges, first, upon the stratum of air which intervenes between the face of the condenser and the slide. The rays, which impinge upon this at an angle greater than the critical angle for glass and air (42°), are reflected back by total reflection. The less oblique components of the beam escape from the condenser undergoing refraction and ordinary partial reflection, as shown in the diagram in connexion with the central rays of the system.

These same rays are again reflected and refracted where they enter the slide.

They undergo refraction and reflection for the third time where they emerge from the upper surface of the cover glass.

Finally, they are refracted and reflected where they enter the objective.

(2) The next case which has to be considered is the case

where the condenser is working through air and the objective is immersed. In this case the last two of the four refracting and reflecting surfaces which came into consideration above are abolished, and the rays follow the simpler course delineated in Fig. 85.

In this, as in the last case, the numerical aperture of the beam, which is furnished by the condenser to the objective, is limited by the total reflection of those components of the beam which

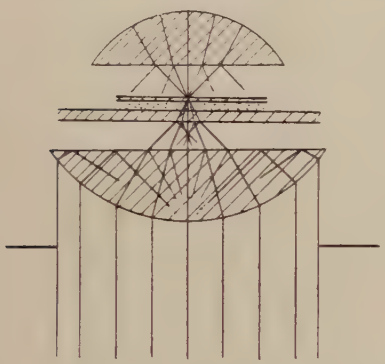


FIG. 84

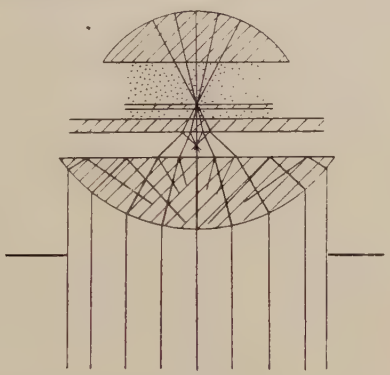


FIG. 85.

impinge at an angle (42°) larger than the critical angle upon the stratum of air which intervenes between the face of the condenser and the slide.

(3) Where the condenser is immersed and the objective is working through air, the N.A. of the beam which enters the objective is limited (as shown in Fig. 86) by total reflection of

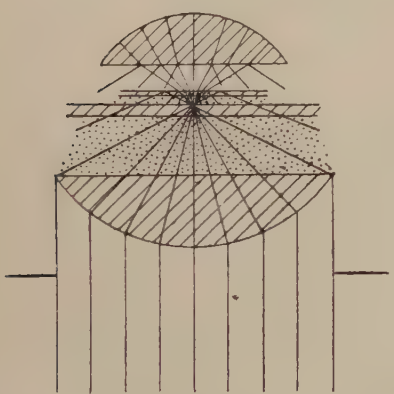


FIG. 86.

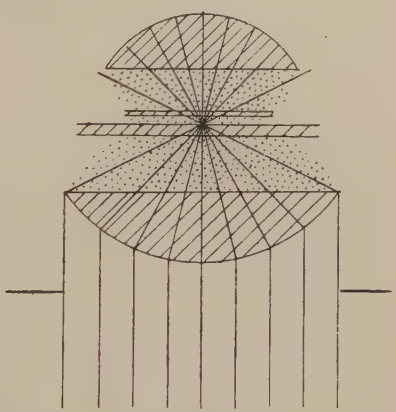


FIG. 87.

those components of the beam which impinge at an angle greater than the critical angle for glass and air upon the layer of air which overlies the cover glass.

(4) Finally, where, as in Fig. 87, both condenser and objective are immersed, the whole beam passes unmutilated and undeflected from condenser to objective, and the numerical aperture of the beam, which comes to focus in the microscopic preparation and which passes thence to the objective, corresponds to the full numerical aperture of the condenser.

18. Distortion of the configuration of the closing limb of the condenser vista and the opening limb of the objective vista by the slide and cover glass and mounting medium.

In the case where the condenser and objective are both working through air, and are both focussed upon a microscopical object, mounted between a slide and cover glass in a medium possessing a lower refractive index than glass, the slide constitutes an active optical element in the closing limb of the condenser vista, and the cover glass, an active optical element in the opening limb of the objective vista, and each of these optical elements changes the configuration of the transmitted beam.

In the case where the microscopic object is mounted in a medium, such as Canada balsam, which possesses a refractive index equal to that of glass, while the objective and the condenser are, as before, working through air, the slide and cover glass fall out of consideration as optical elements, and there comes into account only the aggregate thickness of the stratum of highly refracting medium

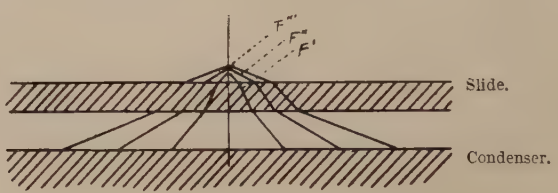


Fig. 88.

(glass supplemented by Canada balsam), which underlies or, as the case may be, overlies the microscopic object.

19. Distortion of the closing limb of the condenser vista by the interposition of the slide in the case where the microscopic object is mounted in air and the condenser is unimmersed.

It will be seen in Fig. 88 that the closing limb of the vista, which would, in the absence of the slide (assuming the condenser to give a corrected image in air), have converged upon a single terminal pole, is here, by the agency of the slide, distorted in such a manner as to come to focus upon a succession of focal points (represented in the diagram by the points F′, F″ and F‴). It is

to be noted with respect to the focal points in question : (*a*) that they lie in each case farther from the condenser than the focus of the undistorted beam ; further, (*b*) that they lie vertically one above the other, the focal point for the delimiting rays of the beam being in each case more remote from the condenser than the focal points for the less oblique components of the beam.

On referring back to *Cap.* VIII, and on comparing Fig. 38, which exhibits the spherical aberration as it occurs in connexion with an uncorrected lens, with the figure now before us, it will be seen that the conditions are exactly reversed,—the delimiting rays of the beam being here brought to a more remote focus (F‴), instead of to a nearer focus, while the more internal rays are here brought to nearer foci (F″ and F′) instead of as there to a more remote focus. It follows that the slide will operate in the direction of correcting spherical aberration in the under-corrected condenser and of over-correcting such aberration in the case where the condenser is designed to furnish a corrected image in air.

20. Distortion of the opening limb of the objective vista by the interposition of the cover glass, in the case where the microscopic object is mounted in air and the objective is unimmersed.

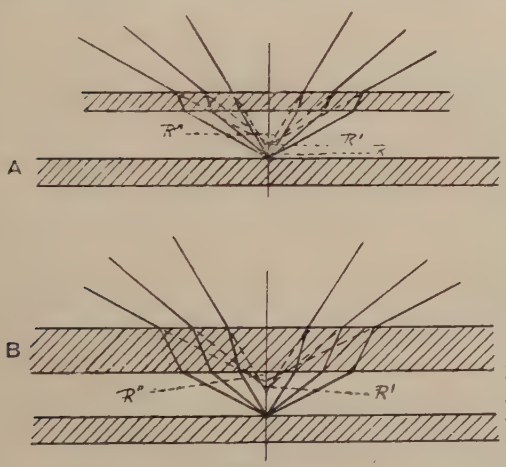


Fig. 89.

It will be seen in Fig. 89, A and B, that a beam, taking origin in air from any radiant point R on the stage of the microscope, is, by refraction in the cover glass, broken up into a composite of beams, such as would be obtained from a series of radiant points R′, and R″ positioned nearer to the objective than the point R, and disposed vertically one over the other in such a manner that the point of origin of the delimiting rays of the beam lies above the point of origin of the less oblique components of the beam.

On referring again to Fig. 38, A, it will be recognized that the distortion of the opening limb of the vista which is here involved is the exact reverse of the distortion in the closing limb of the

vista which is produced by spherical aberration in the case where an uncorrected lens system is employed.

Further, a comparison of Fig. 89, A and B, will bring out clearly that the distortion of the opening limb of the vista by the cover glass will increase *pari passu* with the thickness of the cover glass.

The importance of the thickness of the cover glass in connexion with the correction of a residuum of spherical aberration in the objective, and the achievement of a critical image, will come up for consideration again in *Cap.* XV.

Experiment 1. Rack up the condenser as far as it will go, so as to bring the image of the window which is formed by the condenser to focus in an optical plane lying superficially to the microscope stage. This done, focus down upon the picture with a low-power objective.

Now introduce a slide between the face of the condenser and the optical plane upon which the image of the window is focussed. Note that the picture now disappears from view and that it can be recovered by racking back the microscope tube or, alternatively, by racking down the condenser.

Experiment 2. Introduce a slide between the objective and the optical plane upon which the image of the window is focussed. Note that again the picture will be lost to view. It will again be recovered by racking back the objective or, alternatively, by racking down the condenser.

21. The objective.

It is the function of the objective, employed in combination with the field lens of the ocular, to bring to focus in the principal focal plane of the eye lens the beams which radiate from the microscopic object disposed upon the stage of the microscope.

The projection of the magnified image upon the focal plane of the eye lens is achieved when, by the adjustment of the position of the microscope tube, the microscopic object has come to occupy an optical plane lying somewhat beyond the principal focus of the objective combination.

22. Classification into dry and immersion objectives, and advantages of the latter in the case where highly magnifying systems are employed.

Objectives are classified as dry and immersion lenses respectively, according as air, or a medium of greater refractive index than air, is to be interposed between the cover glass and the anterior surface of the lens combination.

Where the medium of greater refractive index just referred to is water, the objective is spoken of as a *water immersion;* where the interposed medium is cedar oil, possessing the same refractive

index as glass, the objective is spoken of as an *oil immersion* or a *homogeneous immersion* system.

The advantages gained by the substitution of oil immersion for dry lenses are the following :—

(1) The numerical aperture of the beam which is admitted to the objective is not, in the case of the oil immersion, as it is in the case of the dry lens, limited by total reflection from the stratum of air which is interposed between the cover glass and the face of the objective (*vide subsect.* 17, *supra*, and cf. Figs. 86 and 87).

(2) There is no loss of light by the partial reflection of the transmitted rays (cf. Figs. 85 and 87, with Figs. 84 and 86).

(3) In the case of the oil immersion, a longer working distance between the object and the face of the objective is achieved (cf. Figs. 85 and 87, with Figs. 84 and 86 ; also refer to Fig. 27, *Cap.* VII).

(4) The corrections of the objective are not (as in the case of dry lenses generally, and, in particular, of high-power dry lenses) disturbed by the omission of the cover glass, or by the employment of a cover glass of unsuitable thickness, or by the employment, in the mounting of the object, of a layer of balsam of unsuitable thickness.

23. Method of employing an oil-immersion objective.

To employ an oil-immersion objective, place a drop of the cedar-wood oil specially furnished for the purpose by the optician, on the microscopical object, or, as the case may be, upon the surface of the cover glass with which the object is covered in. Focus down with the coarse adjustment until the face of the objective dips into the oil. Then, placing the eye at the eye-lens of the microscope, focus down with the fine adjustment until the object comes into view.

Where difficulty is experienced in bringing the object into view under an oil-immersion lens, this is generally due :—

(*a*) To an accidental inversion of the microscopic slide and the consequent interposition of the whole thickness of the slide between the microscopic object and the face of the objective.

(*b*) To the employment of too thick a cover glass.

(*c*) To stickiness of the face of the objective or of the face of the slide, and consequent adhesion between these faces.

(*d*) To the intrusion of a bubble of air between the face of the lens and the microscopic preparation.

This last is the commonest source of difficulty. It is due to the engagement of bubbles of air in the viscid cedar oil.

The intrusion of such bubbles can in every case be readily detected by examining with a pocket lens the image of the objective aperture which is furnished at a short distance below the Ramsden disc of the eye lens.

24. Focussing of the objective.

In all but the simplest microscopes a rack-and-pinion adjustment is provided for the rough focussing of the objective, and a fine focussing arrangement for its more accurate adjustment.

The importance of the fine focussing adjustment cannot be too strongly emphasized. In point of fact, success or failure in the detection of fine objects under the microscope depends more than upon anything else upon the realization of the fact that the microscopic object is reproduced in the image, not upon a single optical plane, but upon a whole series of optical planes.

The microscopic image has been compared to an extremely thin optical section of the microscopic preparation. In point of fact, it might more aptly be compared to a whole succession of superimposed serial sections, of which only a single one is in view with any particular adjustment of the focus.

It follows that a search through a microscopical preparation, or through a particular field of the microscope, conducted apart from recourse to the fine adjustment, is a search limited to that one of the series of serial sections which happens with one particular adjustment of the focus to lie in the diaphragm of the eyepiece.

A failure to recognize this, displayed by a refusal to use the fine adjustment in the case of a specimen displayed under a high-power objective, and an insistence that the particular focus at which he finds the microscope " exactly suits his sight," is thus the stigma of the layman or the tyro.

Immediate and constant recourse to the fine adjustment betokens, on the other hand, practical familiarity with that shallowness of focus which is the correlative of the fine resolution in depth obtained in highly magnified images and, in particular, in magnified images furnished by wide-angle objectives.

25. Centring of the objective.

We have spoken above of the centring of the condenser. In undertaking that procedure it was tacitly assumed that the objective was in every case duly centred upon the microscope tube.

In point of fact, there is often some inaccuracy in this respect,

and this may be conspicuously aggravated in the case where a badly centred nosepiece is employed.

Inaccuracy of centring shows itself in the fact that a microscopic element which occupies a particular position—such as the centre of the field—when viewed under one objective occupies, as seen through another objective, a different position, or possibly falls, in the case of a higher power objective, altogether outside the field of view.

Inaccuracy in the centring in the case of objectives shows itself also in the fact that the centring of the condenser requires readjustment when one objective is substituted for another.

Unless in the case where Zeiss' sliding objective changers (*vide infra, subsect.* 41) are employed, the rectification of the centring can only be carried out by the optician.

26. Optical properties of the objective.

In connexion with the objective, we have to consider : (*a*) the focal length ; (*b*) the numerical aperture ; and (*c*) the figuring and the corrections of the lens system. The focal length determines the magnifying power, the two factors last mentioned the quality of the image.

27. Focal length and magnifying power of the objective.

The microscopic objectives which are employed in ordinary work range in focal length between $\frac{1}{16}$ inch (1·5 mm.) and 1 inch (25 mm.). We may express their magnifying power on one or other of two alternative systems. Both these systems, be it noted, are confessedly artificial systems.

(1) We may ascribe to the objective a magnifying power corresponding to the magnification which is achieved in the objective vista.

The adoption of this system involves us in two complications :

(*a*) It involves the ascription of a different magnifying power to the objective according as it is employed with a longer or shorter microscopic tube ; (*b*) it involves the ascription of a different magnifying power to the objective, according as it is employed with this or that ocular—combining it, as the case may be, with a stronger, weaker, or a differently positioned field lens.

(2) We may ascribe to the objective the magnifying power which would accrue to it if used as a doublet with the eye.

The adoption of this system of expressing the magnifying power relieves us from all complications.

In accordance with this principle we should ascribe to objectives of the focal lengths specified below the magnifying powers which are set down in the last column of the Table :—

Focal length of the objective (measured in air).		Distance from eye to near point in unaided vision.		Ratio between focal length of objective and eye as given in columns 1 and 2. Magnifying power of objective.
Inches.	Millimetres.	Inches.	Millimetres.	
$\frac{1}{12}$	2 circ.	10	250	120 (circ)
$\frac{1}{8}$	3	,,	,,	80
$\frac{1}{6}$	4	,,	,,	60
$\frac{1}{4}$	6	,,	,,	40
$\frac{1}{3}$	8	,,	,,	30
$\frac{1}{2}$	12	,,	,,	20
$\frac{2}{3}$	16	,,	,,	15
1	25	,,	,,	10
2	50	,,	,,	5

28. Method of measuring the focal length of the objective.

The focal length and magnifying power of the objective may be most conveniently arrived at by measuring, by means of an eikonometer, the cumulative magnification obtained with the objective working in conjunction with any convenient eyepiece, dividing afterwards the cumulative magnification as thus obtained by the magnifying power of the ocular (measured as described below in *subsect.* 45 *infra*).

Example. The cumulative magnification of the objective and of the eyepiece employed is 500. The separate magnifying power of the ocular, measured as in *subsect.* 45, *infra,* is 4. The separate magnifying power of the objective is thus : $500 \div 4 = 125$, and the focal length of the objective 250 mm. $\div$ 125 = 2 mm.

29. On the numerical aperture of the objective and its influence upon the quality of the microscopic image.

The quality of the image depends, as we have seen in connexion with the unaided eye, on the one hand, upon the numerical aperture ; and, on the other hand, upon accurate figuring, correction, and, of course, focussing of the lens system. Large numerical aperture carries with it, in the case of the microscopic objective, the following advantages :—

(1) It conduces to the achievement of finer resolution in depth.

(2) It permits of the imaging of microscopic elements which

are screened from direct view by overlying elements in the preparation.

(3) It reduces the dimensions of the antipoint and thereby gives better resolution in plan.

(4) It permits obliquely incident beams from outlying regions of the field of vision to pass comparatively unmutilated through the objective.

(5) It makes it possible to achieve high magnification, apart from such restriction of the terminal beam as would entail the obfuscation of the image by shadows of intra-ocular and extra-ocular opacities.

In connexion with this last, we may note that where the numerical aperture of the beam which enters the eye is reduced to 0·001, we have, as we have seen in *Cap.* X, *subsect.* 9, conditions which are inconsistent with the realization in the eye of a satisfactory image.

In conformity with this, the maximum magnification which can advantageously be exacted from an objective is a magnification which stops short of reducing the numerical aperture of the terminal beam beyond this limit.

What that maximum magnification is for microscopic objectives possessing numerical apertures of between 1·4 and 0·3, will be learned from the Table below :—

Numerical Aperture of the Objective.	Factor by which that N.A. can be divided before the terminal beam is restricted to 0·001, and (in correspondence with this) maximum magnification which can advantageously be exacted from the lens.
1·4	1400
1·3	1300
1·2	1200
1·1	1100
1·	1000
0·9	900
0·8	800
0·7	700
0·6	600
0·5	500
0·4	400
0·3	300

30. Measurement of the numerical aperture of the objective.

The numerical aperture of the objective can be measured in two alternative ways :—

(1) We may employ an apertometer, such as that designed by Abbe, which measures the numerical aperture of the beam which is projected upon the stage and which passes thence into the objective. By this method we determine the numerical aperture of the opening angle of the objective vista by measuring the numerical aperture of the closing angle of a preceding vista.

(2) We may arrive at the numerical aperture by measuring both the focal length and the Ramsden disc of the objective ; or, in the case where the focal length is known, by measuring the Ramsden disc.

31. Principle of the Abbe apertometer.

The Abbe apertometer is, as will be seen on referring to Plate XII, Fig. 3, A and B, constructed out of a segment of a glass disc. The convex aspect of the disc, which occupies a vertical position as the apertometer lies upon the table or upon the stage of the microscope, constitutes a cylindrical, lenticular window, which admits light to the interior and directs it to a reflecting surface, which is formed by grinding off the inferior and posterior angle of the semicircular glass plate. This reflecting surface deflects the light in such a manner as to send it upwards through a small aperture in the silvered disc. The adjustable metallic wings serve for opening up or shutting down the aforementioned lenticular window in such a manner as to transmit, through the aperture in the silvered disc, a beam of larger or, as the case may be, smaller numerical aperture.

32. Procedure for measuring the numerical aperture of the objective by means of the Abbe apertometer.

Place the apertometer on the stage of the microscope and, employing the lowest available eyepiece, focus down upon it with the objective whose numerical aperture is to be measured. Illuminate the anterior curved face of the apertometer and bring into view under a pocket lens the image of the back of the objective, which is formed in the Ramsden disc of the eye lens. Keeping the Ramsden disc of the objective under observation, adjust now the metallic wings of the apertometer until their edges coincide accurately with those of the edges of the aperture of the objective. When this position has been found, read off the N.A. on the scale inscribed on the upper face of the apertometer. This will represent the angular aperture of the closing angle of the apertometer vista, and at the same time the N.A. which we here require—i.e. the numerical aperture of the opening angle of the objective vista,

in the case where this occupies the full aperture of the objective.

33. Procedure for measuring the numerical aperture of the objective without recourse to the apertometer.

Where the N.A. of the objective is to be measured, without recourse to the apertometer, we require, as has already been indicated above, to know the focal length of the objective combination and the semi-diameter of its Ramsden disc.

The focal length, if not already known, may be measured, as explained in *subsect.* 27, *supra.*

The semi-diameter of the Ramsden disc may be obtained by measuring the back lens of the objective. In the case where the back lens is accessible, as it often is in the case of lower power objectives, a measuring scale may be directly applied. In the case where the back of the lens is sunk in the objective mount, we may conveniently measure the diameter of the image of the fully lit up back lens of the objective by applying our scale at the Ramsden disc of the eye lens and reading it off by the aid of a pocket lens. The measurement thus obtained, multiplied by the magnifying power of the ocular (see under this, *infra, subsect.* 41), will furnish the diameter of the Ramsden disc of the objective.[1]

Example 1. The diameter of the Ramsden disc or "*pupil of entrance*" of a Zeiss 16-mm. dry apochromatic, measured by laying a millimetre scale across the back lens of the combination, is 10 mm. approximately.

The approximate N.A. as deduced from this measurement and the focal length of the objective is $\frac{5}{16} = 0{\cdot}31$.

The N.A. inscribed by the mount by Zeiss is 0·3.

Example 2. The diameter of the Ramsden disc of an 8-mm. Zeiss apochromatic objective (obtained by reading off with a pocket lens a system of transparent micrometrical rulings imposed upon the Ramsden disc of the eye lens of an ocular which magnifies 4 times, and multiplying the obtained reading by 4) is 10.

The approximate N.A. of the objective as deduced from this measurement and the focal length of the objective is $\frac{5}{8} = 0{\cdot}625$ mm.

The N.A. inscribed on the mount by Zeiss is 0·65

Example 3. The diameter of the "pupil of entrance" of a Zeiss apochromatic 3-mm.[2] oil immersion (obtained as in Example 2) is approximately 8 mm.

The approximate N.A. deduced from this measurement and the focal length of the objective is $\frac{4}{3} = 1{\cdot}35$.

The N.A. inscribed by Zeiss upon the mount is 1·4.

[1] The filling in of the aperture of the objective will (as explained in *subsecs.* 11 and 17), in the case of an oil-immersion lens, practically always involve the opening of the substage diaphragm to its widest extent, and the filling in with cedar oil of the interspace between the front face of the condenser and the face of the slide.

[2] This is the equivalent focal length as measured in air.

34. On the accurate figuring and correction of the objective and on the influence exerted by these upon the quality of the microscopic image.

The rôle played by the accurate figuring and accurate correction of the objective in connexion with the quality of the microscopic image will perhaps be most clearly impressed on the mind if we realize that fallings short in these respects exert upon the microscopic image an effect which is comparable to that which is exerted by " errors of refraction " upon the quality of the retinal image in connexion with ordinary vision. In each case—and the same would, of course, apply also to the case of the inaccurate focussing of the microscope—resolution would be impaired by diffusion—the dioptric beams being laid down in the image in the form of diffusion discs, instead of in the form of points.

35. Corrections of the objective.

The following are the more important points to be noted in connexion with the corrections of the objective :—

(1) In every case the objective is corrected in such a manner as to give its best image in the case where it is employed in combination with the field lens of an ocular inserted into a microscope tube of standard length. This standard tube length is 250 mm.[1] (10 inches) in the case of the original long English model ; 160 mm. in the case of the shorter—so-called Continental—model.

In view of the practically universal employment of nosepieces, or other objective changers, the corrections of the objective are now frequently, in the case of the shorter model of microscope, made for a total tube length of 170 mm.

(2) The corrections adopted in an objective represent in every case a compromise—it being necessary to forego such optical perfection as might be attainable along one particular direction, in order to achieve the best general effect.

(3) In the case of the ordinary so-called *achromatic* objectives, what is aimed at in the matter of the correction of chromatic aberration is the bringing together into a single focal plane of the foci of the red and blue rays, i.e. of the rays from either end of the spectrum. In the *apochromatic* objectives of Zeiss, what is aimed at is the bringing together into a single focal plane also of the foci of the rays of an intermediate wave length.

[1] It is to be observed that the tube length is always measured from the back of the objective to the eye lens of the ocular.

(4) The corrections are, in the case of the ordinary achromatic oil-immersion objective, complete—in the sense that the system is not designedly over- or under-corrected.

(5) In the case of the apochromatic objectives of Zeiss, the objective system is designedly over-corrected in such a manner as to allow of the supplementation of the objective by special under-compensated oculars, technically known as *compensation oculars.*

(6) All dry objectives of moderate power are designedly somewhat under-corrected. The object in view is the achievement of perfect correction in the case where the microscopic object is covered in by a cover glass or highly refracting mounting medium of appropriate thickness.[1]

(7) In the case of high-power dry objectives, the responsibility for the accurate adjustment of the corrections to the particular thickness of the cover glass is imposed upon the microscopist. This adjustment is effected either by shortening or lengthening the tube length, or by altering the distance between the lenses of the objective, by means of the correction collar.

36. Correction collar.

Plate XII, Fig. 4, exhibits the mechanical arrangement which is fitted to all high-class, high-power, dry objectives, for the purpose of bringing together or spacing out the optical components of the objective in such a manner as to correct for variations in the thickness of the cover glasses employed. The adjustment of the position of the lenses is in each case made by trial and error, that position of the correction collar being chosen which furnishes in the particular case the most satisfactory image.

In default of a correction collar, a useful adjustment may often be made by separating or, as the case may be, bringing closer together the distal and proximal elements in the objective combination by unscrewing or, as the case may be, tightening up the head of the lower cell.

37. Preliminary considerations in connexion with the testing of the accuracy of the figuring and the adequacy of the corrections.

We have seen that we must, in relation with the quality of the

[1] It may be observed in this connexion that it would be desirable that every optician should, as is done by Zeiss in the case of his apochromatic dry objectives, engrave upon the mount of the objective the particular thickness of cover glass with which he desires his lenses to be employed.

microscopic image, direct our attention : (*a*) to the numerical aperture of the objective, and (*b*) to the accuracy of its figuring and the adequacy of its corrections.

The method of measuring the numerical aperture of the objective has been described. It remains to consider how the accuracy of its figuring and the adequacy of its corrections can be put to proof.

These can be *adjudicated upon* by attending to the *quality* of the image which is furnished by the objective under the conditions which allow of the achievement of a "*critical image.*"

Before dealing with the procedure, it will be well to assign to each of the terms in the foregoing sentence a definite signification.

(*a*) By the *quality* of the microscopic image is here to be understood the general sharpness of definition, the flatness of the field, and imaging on one and the same image plane of different coloured points, lying side by side on one and the same object plane.

To take examples :—

Where the image of a hexagon is to be adjudicated upon, the sharpness, or, as the case may be, the rounding off of the angles is to come into consideration.

Where the image of a non-vacuolated, or, as the case may be, vacuolated, bacillus is in question, what is to be adjudicated upon is the sharpness or diffuse character of the external contour, or, as the case may be, of both external and internal contours.

Where an image of a system of rulings is to be adjudicated upon, what we have to consider is whether the lines are sharply defined, and whether they stand out clearly from each other in such a manner as to allow of their separate identification.

Again, when an extended field is to be imaged, the point of importance is whether the whole field can be brought into view without readjustment of the focus.

Lastly, where we have to deal with red-stained and blue-stained bacteria, disposed as in a thin-film preparation in the same optical plane, we have to observe whether the differently coloured objects are disposed in the image on the same focal plane.

(*b*) The conditions referred to above as the conditions which allow of a critical achievement of *a critical image* are the conditions which are set forth at length in *Cap.* XV.

The more important of these are the centring of the condenser, the proper adjustment of the tube length, and the restriction of the magnifying power within the limits which are consistent with the maintenance of a sufficiently large terminal beam.

Inasmuch as under these conditions conspicuous antipoint and obfuscation are for all practical purposes excluded, any want of sharpness in the focussed image would be an indication of *diffusion*

—in other words, an indication of the representation of the object in the image by diffusion discs instead of focal points.

(*c*) The terms *proving* and *adjudicating upon* are here employed with intent to signify that the accuracy of the figuring and the adequacy of the corrections are estimated by a method of appraisement, and not, as in the case of the numerical aperture, by definite measurement.

The question as to whether a particular contour is or is not sufficiently sharply defined is a matter for skilled judgment. And this same factor of judgment comes into play even in the case where it is a question of deciding whether the individual lines in a system of rulings are sufficiently well defined to allow of separate identification.

38. Procedure to be adopted in connexion with the testing of an objective.

For the testing of an objective we require to have at disposal, on the one hand, a series of suitable test-objects, and, on the other hand, for the purposes of comparison, an objective of approved excellence and of similar numerical aperture.

Further, we require to make, both in the case of the objective which is under trial, and in the case of the objective which furnishes the standard of comparison, the adjustments which are essential for the achievement of a critical image.

Lastly, we require to employ, in testing the objectives in each case, an ocular of similar magnifying power.

The adjustments which must be made in order to obtain in each case a critical image are set forth at length in *Cap.* XV. The question as to what are the most suitable test objects to employ is considered in the following paragraphs.

39. Consideration of the suitability of diatoms as test objects for the proving of objectives.

The test objects which are ordinarily supplied for the proving of microscopic objectives are the finely ridged and indented silicious tests of diatoms.

The employment of these for testing the accuracy of the figuring and the adequacy of the corrections of objectives is open to objection on the ground that the difficulties which stand in the way of the resolution of these test objects are, in many cases, difficulties in connexion with misprision of focus and in connexion with resolution in depth—difficulties, in short, which cannot be surmounted merely by accurate figuring and correction of the objectives.

The difficulties in regard to misprision of focus arise in association with the circumstance that, in connexion with the regular geometrical pattern of the surface markings of diatoms, there may be developed by the interlacement of the rays which have been reflected and refracted in the course of their passage through the object, an equally regular derivative pattern which will lie superficially to the object (*vide Cap.* I. *subsect.* 7, and Plate II, *c*).

The difficulties in connexion with resolution in depth are due, on the one hand, to the above-mentioned phantom pattern, and, on the other hand, to the circumstance that we have, in the case of diatoms, to deal with two superposed systems of markings—one upon the upper, the other upon the lower test.

40. Employment of stained film preparations of bacteria (*a*) as test objects for the general accuracy of the figuring and corrections, (*b*) as special test objects for proving the chromatic corrections, and (*c*) as test objects for proving the flatness of the image field.

Both the difficulties in connexion with misprision of focus and the difficulties in connexion with resolution in depth, which have been adverted to in the previous section, can be avoided by the employment of test objects, in which the elements are disposed irregularly, and in which these are all disposed upon one and the same optical plane.

Test objects of this kind are to hand in the form of stained film preparations of bacterial cultures.

Film preparations of tubercle bacilli which show the vacuolated structure are eminently suitable objects for the testing of oil-immersion lenses.

Film preparations of the micrococcus Melitensis, owing to the minute size of this micro-organism, and of the bacillus pestis, by virtue of the fact that this micro-organism is both small and vacuolated, furnish also very critical test-objects.

For the purpose of testing the accuracy of the chromatic corrections, a film preparation of tubercle bacilli mixed with other bacteria, stained differentially with carbol fuchsin and methylene blue (as in Plate II, *d*), will be found to furnish a very suitable test object. Where the objective is under-corrected, the red-stained tubercle bacilli will be imaged on a plane superficial to that upon which the blue-stained bacteria are brought to focus.

For the purpose of testing the flatness of the microscopic field, any of these preparations are also admirably adapted. In each case, where we have to deal with any appreciable curvature of the

field, the micro-organisms on the periphery of the field will be thrown out of focus.

41. Objective changers.

The rapid substitution of a lower for a higher power objective, or vice versâ, is provided for by the fitting to the microscope tube a nosepiece or a sliding objective changer. (Plate XII, Fig. 2, A and B.)

The sliding objective changers have over the ordinary nosepiece the advantage that they allow of the easy rectification of the centring of each objective. They have also an advantage in the respect that the end of the microscope is not encumbered by the objectives which are for the moment out of use.

42. Barrel of the microscope and draw tube.

The objective is, as we have seen, corrected in such a manner as to furnish a satisfactory image only at a particular conjugate focal distance, which is in each case fixed by the tube length of the microscope.

In the Continental pattern of microscope a tube length of 160 mm., in the original English pattern of microscope a tube length of 250 mm., is adopted as the standard.

The barrel of the microscope is ordinarily somewhat shorter than this, in order to allow for the extension effected by the fitting of an objective changer ; further, to make it possible to employ a curtailed focal length in the case where the preparation happens to be covered in by too thin a cover glass.

A draw-tube fitted within the barrel allows of the tube length being extended (*a*) in the case where the barrel and objective changer, taken together, fall short of the standard tube length ; and (*b*) where too thick a cover glass has been employed.

A millimetre scale is inscribed upon the draw-tube in order to facilitate the correct adjustment of the tube length.

In addition to furnishing the means for obtaining the most satisfactorily corrected image, the draw-tube furnishes also the means for increasing the magnifying power by lengthening out the closing limb of the objective vista.

This method of increasing the magnification is, in point of fact, little to be recommended. It involves, as consideration will show, a sacrifice of the corrections.

43. Width of the barrel.

The width of the barrel exercises a determining influence upon the extent of the field of view.

Of two microscopes possessing the same tube length, the microscope with the wider tube will be found to give the wider field of view. This is, as consideration will show, due to the circumstance that the obliquely incident beams from the periphery of the field are, in the case of the narrower tube, quenched against the sides before they arrive at the field lens of the ocular.

44. The ocular.

Huyghenian ocular.—The ordinary or Huyghenian ocular, which is employed in connexion with the microscope, consists of two plano-convex lenses, disposed with their convex surfaces directed downwards towards the objective. The distal or field lens of the combination works, as already explained, as a doublet with the objective, shaping the divergent beams which proceed from the objective into incurved vistas.

The field lens effects in this way an extension of the field of view, a corresponding reduction in the magnification, and further a flattening of the field.

The proximal element in the combination—the eye lens—works as a doublet with the optical system of the eye, and magnifies the picture which is focussed by the field lens in the diaphragm of the eyepiece.

The effect of the two elements working together is, in every case, a magnifying effect.

Ramsden ocular. A Ramsden ocular consists of two plano-convex lenses, arranged with their convex surfaces facing each other.

Such oculars are employed only for special purposes, in particular, in connexion with eyepiece micrometers, where they have an advantage over other oculars in the respect of furnishing a more accurately corrected image of the scale which is disposed in the plane of origin of the eye-lens vista.

45. Determination of the magnifying power of the ocular.

The most convenient procedure for determining the magnifying power of the ocular, taken as a whole, is the following :—

Either dismount the objective and then measure the diameter of the back lens, and then fit it again to the barrel of the microscope ; or, better, discard the objective and measure the lumen of the lower aperture of the barrel of the microscope.

Insert now into the upper end of the barrel of the microscope the ocular whose magnifying power is to be measured. Extend the draw-tube until the combined total length of the barrel and draw-

tube conforms to the standard length of 160, 170, or, as the case may be, 250 mm.

Taking a pocket lens in front of the eye, and bending down over the eye-lens, identify the image of the back lens of the objective, which is formed in the Ramsden disc of the eye lens, or, as the case may be, the image of the lower aperture of the microscope. Bring accurately into the same optical plane with the image a measuring scale—either a transparent micrometrical scale, such as would be furnished by the diffraction grating (ruled 16 lines to the millimetre) supplied with this book, or, failing this, any ordinary finely divided measuring scale. Now read off the number of divisions which correspond to the image.

The quotient obtained by dividing this measurement into the measurement of the back of the objective, or, as the case may be, of the lower aperture of the barrel of the microscope, will represent the magnifying power of the ocular in the case where employed in a microscope tube of the standard length here in question.

Example 1. The lumen of the lower aperture of the barrel of the microscope measures 2 cm.

The image of this aperture formed by the ocular employed in a tube of 160 mm. length measures 0·5 cm.

The magnification effected by the ocular employed in a tube of 160 mm. length is a 4-fold magnification.

Example 2. The lumen of the lower aperture of the barrel of the microscope measures 1·5 cm.

The image of this aperture, formed by the ocular employed in a tube of 250 mm. length, measures 0·3.

The magnification effected by the ocular employed in a tube of 250 mm. is a 5-fold magnification.

Example 3. The back lens of the objective measures 1 cm.

Its image in the Ramsden disc of the eye lens measures 1·25 mm.

The magnification effected by the ocular is an 8-fold magnification.

CHAPTER XIV.

ON THE INSTRUMENTAL ADJUSTMENTS REQUIRED FOR THE ACHIEVEMENT OF DARK-GROUND ILLUMINATION AND STEREOSCOPIC ILLUMINATION IN PARTICULAR IN CONNEXION WITH WIDE-ANGLED HIGH-POWER OBJECTIVES.

Introductory, and experiments illustrating the influence of the N.A. of the objective on the type of picture imaged in the microscope—Problem of achieving dark-ground illumination in connexion with both wide- and narrow-angled objectives—Two distinct schemes of illumination can be exploited for the purpose of achieving dark-ground illumination—Method of achieving dark-ground illumination in connexion with narrow-angled low-power objectives by the "arrangement by which the preparation is illuminated by solid, and imaged by hollow beams"—Method of achieving dark-ground illumination in connexion with wide-angled high-power objectives by the "arrangement by which the preparation is illuminated by hollow, and imaged by solid beams"—Method of Siedentopf and Zsigmondi—Method of achieving dark-ground illumination in connexion with wide-angled high-power objectives by the "arrangement by which the preparation is illuminated by solid, and imaged by hollow beams."

1. Introductory, and experiments illustrating the influence of the numerical aperture of the objective on the type of picture imaged in the microscope.

We saw in connexion with the experiments in *Cap.* II, *sects.* I and II, that, when the plane of origin of the objective vista is (as in Figs. 72 and 73) occupied by luminous points which radiate light along the optical axis of the microscope and outwards within the compass of a small angle from that axis, the field of the microscopic image is bright, and every microscopic object, which

is enveloped in a medium of lower refractive index, is delineated by a dark outline.

We saw, further, that where the plane of origin of the objective vista is (as in Fig. 76) occupied by a system of luminous points which radiate light through a small angle along an axis disposed obliquely to the optical axis of the microscope, the field of the image is faintly illuminated, while microscopic objects, where these are enveloped in a medium of lower refractive index, are lit up on one side and shaded on the other in such a manner as to furnish a stereoscopic picture (Plate III, *c*).

Again, we saw that where the plane of origin of the objective vista is (as in Fig. 75) occupied by a system of luminous points radiating light in each case in the form of a widely open, hollow cone, the field of the microscopic image is dark, while the microscopic objects, in the case where these are enveloped in a medium of lower refractive index, are delineated by bright outlines (Plate II, *b*).

Finally, we saw that where the plane of origin of the objective vista is (as in Plate XI, Fig. 1) occupied by luminous points which radiate blue light along the optical axis and within the compass of a small angle from that axis, and red light along paths lying very oblique to that axis, the field of the microscopic image is blue, while the microscopic objects in the case where these are enveloped in a medium of lower refractive index are delineated by red outlines (Plate II, *h* and *m*).

In the experiments which have been in question the radiant points were, it is to be noted, viewed either by the unaided eye or, in the case where the microscope was employed, by a narrow-angled objective. In correspondence with this, the rays which came off from the object at a considerable angle to the optical axis were in each case excluded from participation in the image, and only those rays came under consideration which fell within the narrow aperture of the eye, or, as the case might be, of the narrow-angled objective employed.

We have now to realize, by the help of a few simple experiments : (1) that the dispositions adopted in *Cap.* II for the development of the object pictures above referred to give entirely different images in the case where we view the radiant points through wide- instead of narrow-angled objectives ; and (2) that special dispositions have to be made in the case where we desire to achieve in connexion with wide-angled objectives

either *dark-ground illumination*, or *stereoscopic illumination*, or, as the case may be, *illumination by differentially coloured light*.

Experiment 1. Mount a few strands of glass wool in water on a slide and cover in with a cover glass. Having placed the preparation on the stage of the microscope, and having brought it into view with any narrow-angled objective, focus a wide-angled condenser upon it and block out the central light by the aid of a central spot stop such as will just shut off all the light from the field.[1]

The glass strands will now be defined by bright outlines upon a dark field.

Substitute for the low-power objective a shorter-focussed and proportionately wider-angled objective. It will be found that the field has now become bright and that the filaments are delineated upon that field by moderately dark and more or less diffuse outlines.

Comment. Consideration will show that the light which here comes off from each radiant point in the field of the preparation in the form of a hollow cone of light will now be received into the objective. In conformity with this gathering in of the light into the image, the field, which was seen by the narrow-angled objective as dark, is by the objective of wider angular aperture seen as bright.

This difference will obtain universally as between two objectives of different N.A. when employed with a wide-angled condenser—the one with the larger N.A. will in each case see dark outlines upon a bright field in the case where the objective with smaller N.A. sees bright outlines upon a dark field.

Experiment 2. Going back again to the narrow-angled objective, remove the central spot stop and place under the fully-focussed open condenser the parti-coloured stop (Plate I, E, 3) already employed in previous experiments.

The strands of glass wool will now be seen as blue objects outlined in red on a blue field.

Substituting once more for the narrow-angled objective a wider-angled objective, it will be noted that field and glass wool alike are, as indicated in Plate II, *l*, of a uniform purple colour.

Comment. The reason of this is to be found in the fact that whereas in the case of the narrow-angled objective only the central blue element of the parti-coloured beam which takes origin on the object-field (Plate XI, Fig. 1) enters the objective, in the case of the wide-angled objective both the red and the blue elements of the beam are received into the objective and are brought to focus by it. In correspondence with this the wide-angled lens sees as purple the field which the narrow-angled lens sees as blue.

[1] Where a stop of the exact dimensions which are required is not to hand, a stop of approximately the correct dimensions may be employed, the illumination being regulated by racking the condenser up, or, as the case may be, down until the point is arrived at at which the light just goes out from the field.

Experiment 3. Replace the glass wool employed in *Experiments* 1 and 2 by a blood film mounted in air. Returning again to the narrow-angled objective stop down the condenser in such a manner as to project upon the microscopic preparation a beam oblique [1] to the optical axis of the microscope but not sufficiently oblique to fall outside the capacity of the objective.

A picture in relief is now achieved in which the corpuscles stand out stereoscopically from the bright background (Plate III, *c*).

Substitute again for the narrow-angled a wide-angled objective. A dark outline picture will now replace the picture in relief.

It will have been realized by the aid of the above experiments that the character of the picture alters with the capacity of the eye or objective that views it. It will further be plain to consideration that it would be possible to develop on the stage of the microscope a system of radiant points giving off light at an angle such as would furnish when viewed by a series of three objectives of progressively increasing numerical aperture, with the narrowest, dark-ground illumination ; with the one of intermediate numerical aperture, a picture in relief ; and with the widest-angled objective, dark outlines on a bright background.

2. Problem of achieving dark-ground illumination in connexion with both wide- and narrow-angled objectives.

From what has been said above, the reader will immediately discern that as soon as we come to deal with high-power wide-angled objectives, and in particular with oil immersion objectives the achievement of dark-ground illumination is no longer the perfectly simple affair which it is when we are dealing with low powers and narrow-angled objectives.

It will, however, be possible to achieve dark-ground illumination in connexion with all objectives without exception, provided that the general principles which come into consideration in connexion with this form of illumination are clearly apprehended.

3. Two distinct schemes of illumination can be exploited for the purpose of achieving dark-ground illumination.

Dark-ground illumination can be achieved under two different conditions :—

[1] A beam of the desired obliquity can be obtained by closing the sub-stage diaphragm and then displacing it outwards in such a manner as to dispose the aperture in the diaphragm (as in Plate XII, Fig. 1) under periphery of the condenser.

(1) Where the condenser furnishes a system of hollow beams which go wide of the aperture of the objective except in so far as these are deflected into that aperture by the microscopic object.

(2) Where the condenser furnishes a system of solid beams which traverse the central region of the objective except in so far as these are by the agency of the microscopic object deflected into the outlying regions of the lens.

Here provision must be made for blocking the undeflected beams so that the deflected light alone shall contribute to the image.

Figs. 90 and 91 will serve to explain the two schemes of illumination which are here in question.

In Fig. 90 we have the first arrangement—the arrangement with which the reader is already familiar. We are dealing here with a condenser whose numerical aperture exceeds that of the objective, and a central spot stop which lies in the apertural plane of the condenser vista, and which cuts out from each beam all that central segment which would normally enter the aperture of the objective. By this arrangement the beams which pass through the microscopical preparation without impinging upon a microscopic object go wide of the aperture of the objective—giving us a dark field; while every beam which impinges upon a refracting and reflecting element is deflected into the objective—giving us a bright image upon the dark field.

We may conveniently speak of this arrangement as the *arrangement by which the preparation is illuminated by hollow, and imaged by solid, beams.*

In Fig. 91 we have a wide-angled objective employed in combination with a condenser which is stopped down in such a manner as to transmit beams which will occupy the centre only of the apertural plane of the objective. Upon the stage we have, again, an object which deflects the beam, spreading it out here so as to occupy also the outlying zone of the objective.

The diagram shows that a central spot stop, if disposed, either, as at B, in the apertural plane of the objective vista, or alternatively, as at A, in the apertural plane of the eye vista, will block out all the light from the field, leaving unobstructed only such light from the object as passes through the peripheral zone of the objective. By the agency of this light a bright image is obtained upon a dark field.

We may conveniently speak of this arrangement as the *arrangement by which the preparation is illuminated by solid, and imaged by hollow, beams.*

FIG. 90.
ARRANGEMENT BY WHICH THE PREPARATION IS ILLUMINATED BY HOLLOW, AND IMAGED BY SOLID, BEAMS.

FIG. 91.
ARRANGEMENT BY WHICH THE PREPARATION IS ILLUMINATED BY SOLID, AND IMAGED BY HOLLOW, BEAMS.

It is open to us to exploit in connexion with any type or objective, according as may be more convenient, either the one or the other of these schemes of illumination.

4. Method of achieving dark-ground illumination in connexion with narrow-angled low-power objectives by the "arrangement by which the preparation is illuminated by solid, and imaged by hollow, beams."

The reader has already familiarized himself with the former of the schemes of illumination which have been in question above. He will be well advised—in spite of the consideration that the scheme of illumination with which he is already familiar is in point of fact the more convenient arrangement to adopt in connexion with low-power, narrow-angled objectives—to experiment in connexion with this type of objective also with the alternative scheme of illumination.

Experiment. Place upon the stage of the microscope a fine glass filament or a few strands of glass wool mounted in water under a cover glass. Fit to the microscope a low-power objective and a low-power ocular, and focus down upon the preparation. This done, focus also the condenser upon the preparation. Now removing the eye from the microscope, bring into view with an inspection lens the Ramsden disc of the eye lens and shut down the substage diaphragm, until only the central area of the back of the objective is illuminated.

Having made these adjustments, dip either a glass spherule—obtained as explained in *Cap.* II, *sect.* I, *subsect.* 2—or a circular loop of fine wire into a suspension of Indian ink, and place a series of different-sized drops of the ink on the surface of a carefully cleaned cover glass. Having allowed the ink to dry, bring the cover glass into the plane of the Ramsden disc of the eye lens, and with the aid of the pocket lens adjust matters so that the bright centre of the Ramsden disc may be just occulted by one of the spot stops.

Maintaining the stop accurately in position, bring the eye down into the neighbourhood of the eye lens, and, on looking through the microscope note that the glass filaments are now delineated with bright outlines upon a dark field, instead of betore by dark outlines on a bright ground.

5. Method of achieving dark-ground illumination in connexion with wide-angled high-power objectives by the "arrangement by which the preparation is illuminated by hollow, and imaged by solid, beams."

The conditions which have been set forth above as essential to the achievement of dark-ground illumination by the arrangement here in question can in the case of high-power wide-angled objectives be realized—

(*a*) In the case where the full numerical aperture of the con-

denser exceeds that of the objective and is not already exploited, by exploiting that aperture to the full.

This device is defective in the respect that, as the obtruded segment of each hollow beam becomes larger and larger, an ever smaller segment remains over for the illumination of the object.

(*b*) In the case where the numerical aperture of the condenser is already fully exploited, by cutting down the aperture of the objective by a retro-objective annular stop.

This device is unsatisfactory in the respect that it converts a wide-angled objective into a narrow-angled objective, furnishing in correspondence with this an image which does not present any points of superiority over that which would be furnished by a low-power narrow-angled objective supplemented by a high-power ocular.

Experiment 1. Fitting to a microscope, which is furnished with the ordinary wide-angled Abbe condenser, a high-power wide-angled dry objective, and placing upon the stage of the microscope a few strands of glass wool mounted in water, satisfy yourself that dark-ground illumination cannot be achieved simply by the introduction of a central spot stop into the substage diaphragm.

Now immerse the condenser and verify that dark-ground illumination can under these circumstances be obtained.

Employing a parti-coloured stop in the substage, verify further that what holds true in connexion with dark-ground illumination holds true also of illumination by differentially coloured light.

Experiment 2. Employing the arrangement which was employed at the outset in *Experiment* 1, drop into the mount of the objective, in such a manner that it may come to rest upon the posterior lens of the combination, an annular stop previously cut out of thin cardboard and blackened. It will now be found that a dark-ground illumination is achieved even apart from the immersion of the condenser.

6. Method of Siedentopf and Zsigmondi.

Siedentopf and Zsigmondi have of recent years exploited for the achievement of dark-ground illumination a principle which is in all essential respects identical with that which was in question in *subsect.* 5 (*a*) and *Experiment* 1 *supra*.

The device which is employed is the illumination of the object by a beam of light projected horizontally and brought to focus in the microscopical preparation in the form of a cone or wedge.[1]

There is, as will be clear from the consideration of A and B in

[1] The claim which Siedentopf and Zsigmondi put forward in connexion with their method, that it is a method which reveals to view elements of "ultra-microscopic minuteness," will be considered in *Cap.* XVI, *subsect.* 1.

the figure below, as between this method of dark ground illumination and that obtained by the method which is in question in *subsect.* 5 (*a*) and *Experiment* 1 *supra*, no difference except in respect to the absolute horizontality and the larger aperture of the illuminating beam.

It is to be noted, in particular, that the restriction of the illumination to a single optical plane in the microscopic preparation,

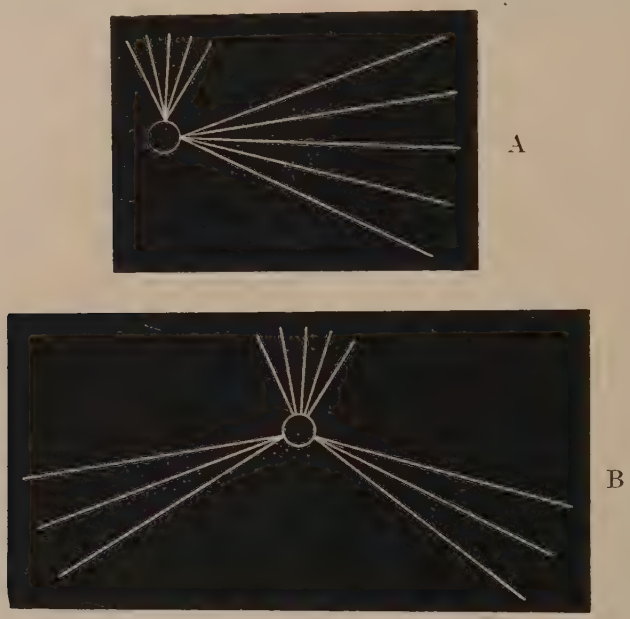


FIG. 92.

A. SCHEME OF DARK-GROUND ILLUMINATION EMPLOYED BY SIEDENTOPF AND ZSIGMONDI.

B. SCHEME OF DARK-GROUND ILLUMINATION WHICH CAN BE OBTAINED WITH A CENTRAL SPOT STOP, IMMERSED WIDE-ANGLED CONDENSER, AND POINT SOURCE OF LIGHT.

which is claimed as a specially distinctive feature of Siedentopf and Zsigmondi's system of illumination, can be obtained also with a central spot stop and condenser in the case where a point source of illumination, and in conformity with this a single illuminating beam, is employed. This will be manifest on considering Figs. 75, 90 and 92 (*B*).

The method of Siedentopf and Zsigmondi involves, as will immediately be appreciated, the employment of complicated and expensive apparatus.

7. Method of achieving darkground illumination in connexion with wide-angled high-power objectives by the "arrangement by which the object is illuminated by solid, and imaged by hollow, beams."

The reader will have appreciated that the arrangement which we may now, in view of the device of Siedentopf and Zsigmondi, speak of in more general terms as "the arrangement by which the object is illuminated by beams which go wide of the aperture of the objective," is in connexion with high-power wide-angled objectives, unsatisfactory. We have seen that it is unsatisfactory, either (*a*) by reason of the amount of light which strikes the object becoming progressively less as a larger and larger segment is cut out from the hollow beams, or (*b*) by reason of the aperture of the objective being cut down, or (*c*) by reason of the complicated and expensive apparatus, as in the method of Siedentopf and Zsigmondi, required for its exploitation.

All these difficulties disappear when we have recourse, in connexion with high-power wide-angled objectives, to the "arrangement by which the preparation is illuminated by solid, and imaged by hollow, beams." In particular the difficulties disappear when we employ that form of the arrangement in which a retro-ocular central spot stop is, as has recently been suggested by Gordon,[1] substituted for the retro-objective central spot stop —already more than once exploited, only to be abandoned as inconvenient.

Owing to the circumstance that the device of the retro-ocular stop has only just been alighted upon, the final form which the apparatus which will be required for the exploitation of the proposed method has not yet been arrived at. It would seem probable that it will take the form of a wheel of central spot stops furnished with a screw movement both in the vertical and horizontal plane, so as to allow of the particular stop which may be required being brought accurately into the centre of the Ramsden disc of the eye lens.

[1] *Journal of the Royal Microscopical Society*, April, 1906.

CHAPTER XV.

ON THE ADJUSTMENTS REQUIRED FOR THE ACHIEVEMENT OF A CRITICAL IMAGE.

Introductory—Necessity for the regulation of the source of illumination—Suggestions for the avoidance of the difficulties which are associated with the employment of an extended source of illumination—Disadvantages which attach to Nelson's method of evading these difficulties—Methods of reducing the dimensions of the light source—Importance of centring the condenser—Principles in accordance with which the working aperture of the condenser ought to be regulated—Method of regulating the substage arrangements in such a manner as to give us a system of homogeneous beams of the greatest possible numerical aperture—Choice of objective—Adjustments which may be required for the purpose of obtaining the full benefit of the optical corrections of the objective—Elimination of bubbles in the immersion medium and of any obtruding obstacles which may be disposed on the upper or lower face of the condenser or objective—Choice of ocular—Experiments exhibiting the effect which is exerted on the microscopic image by the contraction and opening up of the beams which are delivered by the microscope to the eye.

1. Introductory.

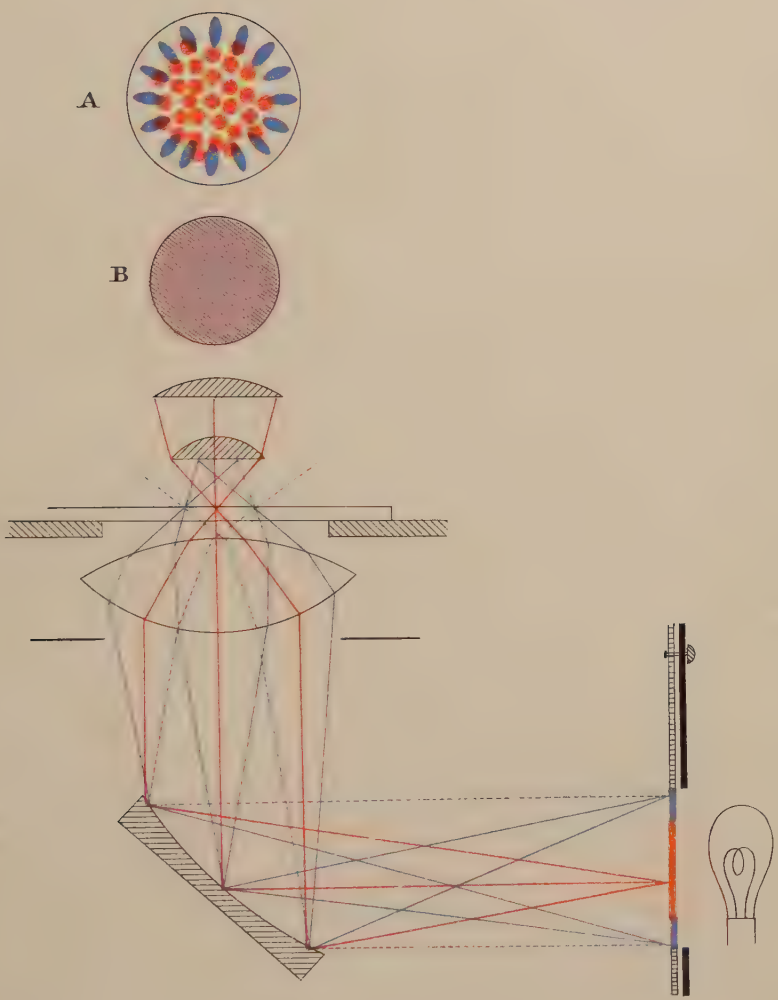
Where we are dealing with an ordinary microscopic object, and where we have at disposal in our microscope a surplus of optical power, the resolution of the object is obtained irrespectively of the achievement of a *critical image.*

Such critical image is, however, indispensable where we have to deal with an object which is only just within the competence of our instrument.

It can be achieved by undertaking the instrumental adjustments which come under consideration in this chapter.

Let us note with respect to these that, while they are of obligation only in the case where we have to resolve a difficult microscopic object, they may none the less advantageously be undertaken as a matter of routine in connexion with microscopic work generally, and, in particular, in connexion with all work with high powers of the microscope.

Plate XIV.



We may conveniently begin with the consideration of the light source.

2. Necessity for the regulation of the source of illumination.

The necessity for the regulation of the source of illumination will appear when we consider the optical conditions which obtain where an extended radiant field such as is furnished by the sky or a broad lamp flame is employed as a source of light. There will be formed in such a case upon the stage of the microscope by the focussed condenser an image of the light source which will extend beyond the limits of the field of view of any objective. From the radiant points included within this illuminated area beams will pass into the aperture of the objective. Those, from the centre of the field—always assuming that their numerical aperture does not exceed the numerical aperture of the objective—will pass through the aperture unmutilated. It will be different with respect to the beams which proceed from the periphery of the field. These, taking the aperture obliquely, will, unless in the case where their numerical aperture is much less than that of the objective, be cut down in an unsymmetrical manner by the margin of the objective, exactly in the same way as would be the case if transmitted through an elliptical, or, in the extreme case, through a slit aperture.

BATTERSEA PUBLIC LIBRARY

It follows that while the radiant points in the centre of the field will be represented in the image by circular antipoints whose dimensions will be determined by the full numerical aperture of the objective, the radiant points on the periphery of the field will be represented in the image by elliptical or linear antipoints whose long axes will in each case be disposed radially to the aperture, overlapping the antipoints in the centre of the field in such a manner as to fog the image.

These are the optical conditions which are represented on Plate XIV, the beams from the centre of the radiant field being here distinguished from those from the periphery by a difference of colour.

Experiment. Placing on the microscope stage under an oil immersion any of the common test diatoms, open wide the substage diaphragm, immerse the condenser, and bring into the field of the mirror the bright flame of a paraffin lamp, disposed broadside on to the microscope.

The conditions will now, except for the absence of the coloured screen, be those represented in Plate XIV. In other words, the image of the light source which is formed on the microscope stage will extend far

beyond the limits of the field of the objective, and from each of the radiant points in that illuminated area beams will be passing into the aperture of the objective.

On looking down the microscope it will now be seen that a fog of light rests over the whole image, muffling all the shadows and destroying all contrast.

The effect on the image will be something like that which would have been produced in Plate II, *e*, if a wash of white had been passed over the whole picture.

3. Suggestions for the avoidance of the difficulties which are associated with the employment of an extended source of illumination.

Two alternative remedies have been proposed for the fogging of the image which was adverted to in the foregoing subsection.

(1) The numerical aperture of the beams which proceed from the stage may, as has been proposed, in particular by Mr. Nelson, be restricted by shutting down the substage condenser until the numerical aperture of these beams becomes considerably less than the numerical aperture of the objective. When this has been done the beams which proceed from the outlying portions of the illuminated area on the stage of the microscope will—the obliquity of their incidence notwithstanding—pass through the aperture of the objective unmutilated.

Experiment. Maintaining the other conditions as in the experiment in the previous subsection, bring into view under an inspection lens the Ramsden disc of the eye lens, and shut down the iris diaphragm until, as the numerical aperture of the beams which proceed from the stage is gradually closed down, the peripheral zone of the back lens of the objective which was before entirely illuminated as in Plate X, Fig. 3 A, falls into shadow as in Plate X, Fig. 3 B, and Plate XV, B.

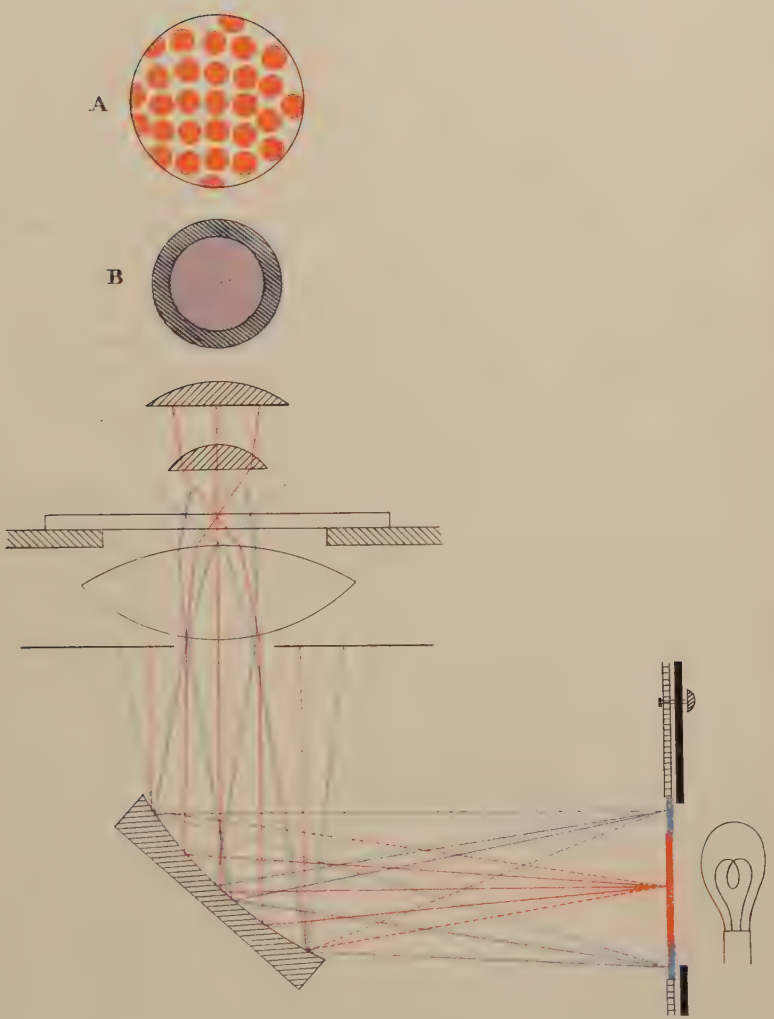
The conditions which will now have been established are those represented in the last-mentioned Plate.

Note that, in association with the establishment of these conditions, the fog of light which before rested over the image has disappeared and that the unilluminated parts of the image come out of a deep black.

(2) The peccant radiant points which lie on the periphery of the field of view and beyond it may, as suggested by Mr. Gordon, be quenched by setting up in front of the light source a screen in such a manner as to restrict the radiant field which sends light to the condenser. Hereby the illuminated area on the microscope stage which radiates into the aperture of the objective will also be restricted.

Experiment. Substituting for the broad flame employed in the experiments above a brilliant point source of light (obtained either

Plate XV.



by the aid of the apparatus described below, or by placing a cardboard screen perforated with a small hole in front of the lamp flame), throw open the substage diaphragm to its fullest extent.

The conditions which will now have been established are those represented in Plate XVI.

Note that with the establishment of these the sharp contrast between dark and bright which was achieved in the last experiment is again obtained.

4. Disadvantages which attach to Nelson's method of evading these difficulties.

It is to be noted with regard to the method of Nelson that its adoption involves a sacrifice of numerical aperture throughout the whole field. The extent of the sacrifice will be realized on viewing the Ramsden disc of the eye-lens through an inspection lens, and reflecting that by the shutting down of the numerical aperture of the transmitted beams, there has in effect been cut off from the aperture of the objective—except only for the purpose of the transmission of the obliquely incident beams—the whole of that peripheral zone of the objective which is in Plate X, Fig. 3, B, and Plate XV, B, occupied by shadow.

In association with this restriction of the beams the radiant points upon the stage will in the image be represented by larger antipoint discs (cf. Plate XV, *a*, with Plate XVI, *a*).

This increase of the dimensions of the antipoint pattern will *pro tanto* interfere with resolution.

5. Methods of reducing the dimensions of the light source.

The simplest method of giving effect to the suggestion that the light source should be restricted is to turn the flame of the microscope lamp edgewise on to the microscope. In this manner we diminish the sectional area of the flame which radiates light to the mirror, correspondingly reduce the illuminated area on the microscope stage, and *pro tanto* overcome the difficulty which arises when beams are projected very obliquely into the aperture of the objective.

But this method suffices only where we are dealing with low-power objectives which cover a comparatively large field. In the case where high objectives are employed, we shall do well, in accordance with the suggestion of Gordon, to still further reduce our radiant field.

This can be effected by placing in front of the lamp a cardboard screen perforated in each case with an appropriate aperture. More

convenient, however, for the regulation of the aperture, is a wheel of stops such as is fitted to the apparatus shown in the figure below.

We have here a Nernst lamp [1] enclosed in a box which is open below, and which is provided with a window (seen in the anterior wall of the box in the reflected image) whose aperture can be regulated by a wheel of stops.

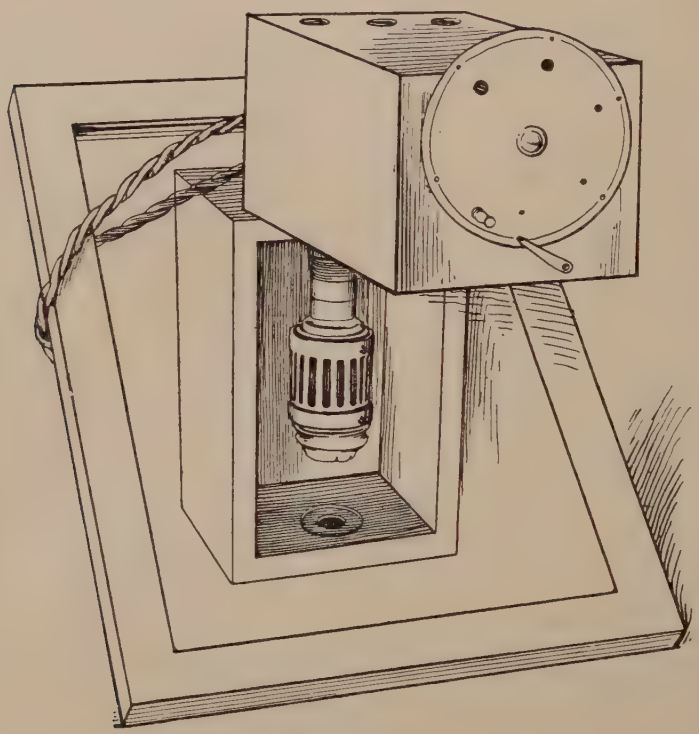


FIG. 93.

Apparatus for the regulation of the illumination, superposed upon a mirror for the display of the internal arrangements.

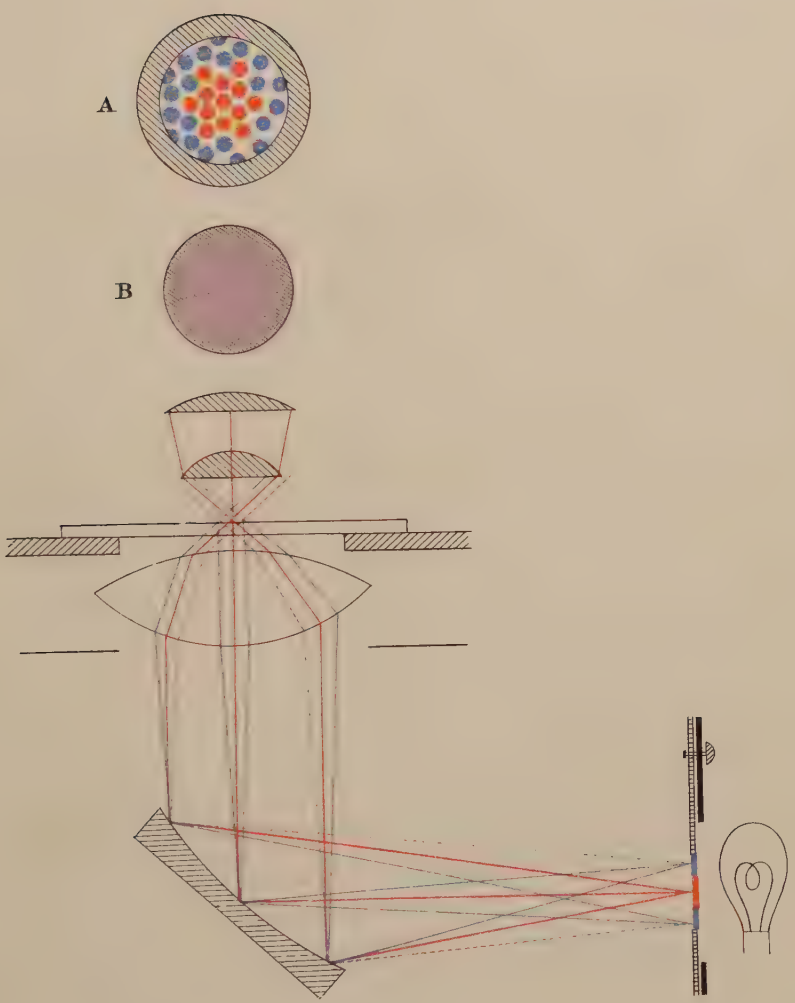
6. Importance of centring the condenser.

After what has been said in *subsect.* 2, *supra*, with respect to the fogging of the image in the case where the beams which traverse the objective are cut down in an unsymmetrical manner in their passage through its aperture, it will be manifest that a critical image can be achieved only when the optical axis of the condenser is brought accurately into allineation with the optical axis of the microscope.

The optical conditions which we have to deal with when the condenser is out of allineation with the objective are set forth on

[1] There may, of course, be substituted for the Nernst lamp as here shown an acetylene lamp, appropriately fitted, or any other brilliant source of light.

Plate XVI.



BATTERSEA

Plate XVII. Here, by consequence of the mutilation of the beams in the aperture of the objective, we have the radiant points upon the stage represented in the image by a system of overlapping eliptical antipoints. Such conditions are incompatible with the satisfactory resolution of any fine object.

The method of centring the condenser has already been described in *Cap.* XIII, *subsect.* 6.

7. Principles in accordance with which the working aperture of the condenser ought to be regulated.

In connexion with the regulation of the numerical aperture of the beams which are furnished by the condenser we have to take into account in each case :—

(1) The effect that the opening up and closing down of these beams will exert on the stage picture.

(2) The effect that such widening and restriction will exert upon the microscopic image.

(1) Inasmuch as the proper development of the microscopic image is conditional upon the proper development of the stage picture, the opening up or restriction of the beams must be considered primarily from the point of view of its effect upon the stage picture.

(2) Only when all the demands of the stage picture have—as we may assume to be the case here—been fully satisfied, are we free to consider the further instrumental adjustments which will be conducive to the development of a critical microscopic image.

(*a*) Our first consideration here must be to avoid the unsymmetrical cutting down of the beams in the aperture of the objective. Where the dimensions of the light source have not been restricted, the numerical aperture of the condenser must—though this will in other respects prejudice the image—be cut down as suggested in *subsect.* 3 (1), *supra.*

(*b*) Where the unsymmetrical cutting down of the beams has already been avoided by the device suggested in *subsect.* 5, our next consideration must be the capacity of the objective for bringing to accurate focus the whole beam which it is capable of admitting. Where the objective does not fulfil this requirement we must again, as in (*a*), cut down the aperture of the condenser in such a manner as to leave the peripheral zone of the back lens of the objective unoccupied.

(*c*) Where the unsymmetrical cutting down of the beams in the aperture of the objective is avoided, and where this

last is capable of dealing in a satisfactory manner with a full beam—and we may assume that these are the conditions with which we have to deal here—we are completely free to comply with the fundamental principle of all critical image formation. That principle is to employ such a system of homogeneous beams as will fill in the aperture of the objective to its fullest possible extent.

8. Method of regulating the substage arrangements in such a manner as to give us a system of homogeneous beams of the greatest possible numerical aperture.

The methods of focussing, immersing, and centring the substage condenser so as to obtain beams of the greatest possible numerical aperture have already been described in *Cap.* XIII, *subsects.* 4–8. These adjustments may not by themselves suffice for the development of the critical image. It may happen, even after they have been made, that examination of the Ramsden disc of the eye lens through an inspection lens may reveal that the light is coming through the back lens of the objective in an unsymmetrical manner —the central area and the outer zone only of the lens being lit up, while the intermediate zones are left in comparative darkness. In such a case only a very blurred microscopic image will be obtained. It will be found that this can be rectified by racking the condenser up or down until a homogeneously illuminated disc of light occupies the back of the objective.

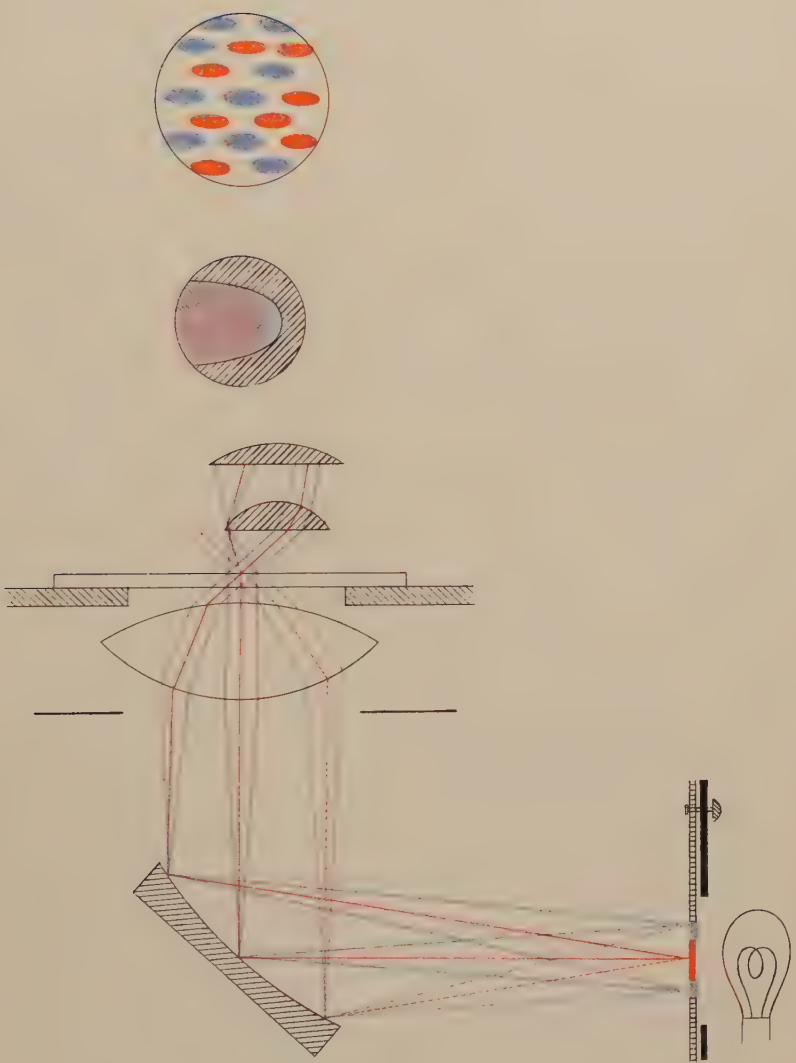
9. Choice of objective.

We have seen that the quality of a lens image depends (*a*) upon the more or less accurate figuring, correction, and adjustment of the system of lenses, which is in question, (*b*) upon the larger or smaller numerical aperture of the opening and closing limbs of such system. We have seen also that the former of these factors is in the case of the objective, as in the case of the unaided eye, the factor of dominant importance.

Without for a moment losing sight of the fact that the importance of aperture is subordinate to that of the other optical characters of the objective, we may here, dismissing from consideration all questions other than those which relate to numerical aperture, consider in connexion with the selection of our objective that particular factor.

The principle which ought to direct our choice in such a case is a very simple one.

Plate XVII.



We ought in each case to select an objective which will admit a beam of maximum numerical aperture, and which will furnish to the eye at the eye lens a beam whose diameter will be equal to, or as little inferior as may be, to the diameter of the pupillary aperture.

The ideal condition of a terminal beam completely filling in the pupillary aperture was the condition which Helmholtz had in view when he spoke of *normal magnification*. This condition will be satisfied only in the case where the numerical aperture of the beam which enters the objective exceeds the numerical aperture of the beam that enters the eye in ordinary vision by a factor equivalent to the magnification of the final image.

Taking cognizance in passing of the fact that this is the principle which the bacteriologist applies when he discards, as he does in his ordinary work, all high-power lenses except oil immersions, we may set before us once more here the advantages which are associated with the employment of objectives of large numerical aperture.

The advantages which are associated with the employment of objectives of large numerical aperture are the following:—

(*a*) The quantity of light which is gathered into the opening limb of the objective vista compensates for the reduction of brilliancy which is entailed by magnification.

(*b*) Elements in the microscopic object which are masked from direct view by interposed elements are imaged by rays which circumvent these interposed elements.

(*c*) Adequate resolution in depth is achieved, in other words, separate and practically unclouded images are obtained of the successive radiant planes which are positioned in the object.

(*d*) The antipoint of the image is kept within moderate dimensions, with the result that adequate resolution in plan is achieved.

(*e*) There is supplied to the eye at the eye lens a beam which is, in the case where excessive magnification is avoided, wide enough to ensure the freedom of the retinal field from obfuscation by the shadows of extra-ocular and intra-ocular opacities.

Example. In the case where a 100-fold magnification is to be exacted in the microscopic image, and where our choice lies between two equally well corrected objectives possessing N.As. of 0·6 and 0·3 respectively, preference ought, other things being equal, to be given to the former. The 0·6 objective would not only gather in more light into the image and give better resolution in depth, but it would give us the required magnification in association with a terminal beam of 0·006 n.a., i.e., a beam which would fill in the whole aperture of the pupil. The 0·3 objective would, in association with a 100-fold magnified image, give us a beam of 0·003 n.a., i.e., a beam whose diameter would be less by one-half than the diameter of the pupil.

10. Adjustments which may be required for the purpose of obtaining the full benefit of the optical corrections of the objective.

We have seen in *Cap.* VIII that the utmost which the optician can hope to achieve by his corrections is the accurate representation of the microscopic object in the case where the image is formed at a conjugate focal distance prearranged by him. We have seen also that the optician stipulates that where a dry lens is employed the microscopic object shall be covered in by a cover glass of a standard thickness.

It falls to the microscopist to see that the conditions which are postulated by the optician are, so far as possible, in each case complied with, and that any departure from these conditions is duly compensated for.

In the case where an immersion lens is employed, or where a dry lens is employed in association with a cover glass of appropriate thickness, the microscopist is not called upon to do anything more than verify that he is employing the correct tube length. If he finds that the distance measured from the back of the objective to the eye lens is less than the correct tube length (in the continental pattern of microscope 160, or, as the case may be, 170 mm.), the microscopist will make the necessary extension of the draw-tube.

In the case where the microscopic object is being viewed by a dry lens through a cover glass which is thicker or thinner than the standard, the microscopist will make the required adjustment of the correction collar, or, as the case may be, of the tube length—proceeding in each case by the method of trial and error until a critical image shall have been attained.

11. Elimination of bubbles in the immersion medium, and of any obtruding obstacles, which may be disposed on the upper or lower face of the condenser or objective.

In view of the possibility of the deterioration of the image by the accumulation of dirt upon the upper or lower surfaces of the objective or condenser, and by the intrusion of air bubbles into the immersion media in the case where the condenser and objective are immersed, it will be well in every case to search with a pocket lens, with a view to detection of obstructing obstacles, the Ramsden disc of the eye lens and the image planes immediately below this.

Where such obstacles are found the remedy immediately suggests itself.

12. Choice of ocular.

The principle of securing the desired cumulative magnification without any unnecessary reduction of the numerical aperture of the beam which comes to focus in the eye, a principle which dictates as we have seen (*subsect.* 9, *supra*), resort to objectives of the widest possible numerical aperture, dictates also, as consideration will show, in each case the employment of the lowest possible ocular which will give the desired magnification.

The diagram below will make clear to the eye that the higher magnification which is obtained by substituting for an eye lens of longer focus an eye lens of shorter focus, in connexion with one and the same objective vista, results, as shown in Fig. 94 below in the delivery to the eye of a narrower beam.

FIG. 94.

13. Experiments exhibiting the effect which is exerted on the microscopic image by the contraction and opening up of the beams which are delivered by the microscope to the eye.

Experiment 1. Fit to the microscope any low-power objective, let us say a 16-mm. objective possessing a N.A. of 0·3, and a low-power ocular, let us say an ocular giving a four-fold magnification, and focus upon a microscopic preparation illuminated by a fully open condenser.

Withdrawing the eye to a distance of 10 inches or more from the eye lens, note that the Ramsden disc of the eye lens is sensibly of the same dimensions as the pupil of the eye.

Take in hand now a piece of somewhat fine wire—say wire of 0·5 mm. diameter—place this transversely across the eye lens and then, bringing the eye up to the eye lens, look down the microscope. The presence of the wire will be notified by a very faint band of shadow which does not block out from view any part of the microscopic field.

Substitute for the low eyepiece an eyepiece possessing a two or three-fold higher magnifying power. Withdrawing the eye to a distance of 10 inches, note that the dimensions of the Ramsden disc are now reduced to a half or, as the case may be, a third of what they previously were. In association with this it will be found on bringing the eye up to the eye lens, and looking down the microscope that the wire when laid across the eye lens will now cast a heavier shadow.

Lastly, extend the draw-tube to its fullest extent and take note of the fact that we have now a further reduction of the dimensions of the

Ramsden disc and a further increase in the depth of the shadow. The shadow will now definitely block out from view a band of the microscopic field.

It will be appreciated that the depth of the shadow is in each case proportionate to the segment of the beam which is cut out by the obtruding wire. In the case where the shadow is absolutely black, the diameter of the beam is less than the diameter of the wire.

Experiment 2. Keeping the high-power ocular in place and the draw-tube extended, substitute for the low-power objective a high-power objective and shut down the diaphragm in the substage in such a manner as to restrict the beam which passes into the objective.

Before focussing down upon the microscopic object note that the Ramsden disc has been reduced to the dimensions of the finest pin point, and then look into the microscope and observe that the blank microscopic field is everywhere clouded with shadows ringed round with diffraction patterns such as those shown on Plate XVIII, A and C. These shadows correspond to particles of dust upon the eye lens and to extra- and intra-ocular opacities in connexion with the eye.

Now push in the draw-tube, focus down upon the microscopic preparation, and open up the substage diaphragm, and notice as you do so that each of these procedures brings about an expansion of the Ramsden disc, and a diminution in the depth of the shadows thrown by the finest class of obstacles. Take, however, note of the fact that larger obstacles such as are constituted by eyelashes or by a wire laid across the eye lens still throw almost absolutely black shadows.

CHAPTER XVI.

ON THE QUESTION AS TO WHETHER THERE ARE DEFINITE LIMITS IMPOSED UPON MICROSCOPIC VISION AND RESOLUTION; AND ON THE REAL NATURE OF THE LIMITATIONS ENCOUNTERED IN CONNEXION WITH THE EMPLOYMENT OF HIGH MAGNIFICATIONS.

Introductory—Theoretical limit of resolution—Question as to whether the limit of Helmholtz is the untransgressable limit of resolution—Question as to the real nature of the limit of resolution which is encountered by the microscopist in connexion with the employment of high magnifications—Is it possible to advance to a higher limit of resolution than that attained by the present optical arrangements?—On Gordon's method of opening up the beam before it is delivered by the microscope to the eye—Appraisement of this achievement—Question as to whether it opens up a prospect of an advance towards increased resolving power.

1. Introductory.

The term *ultra-microscopic* has of late come into current use in connexion with very minute elements which have not as yet been satisfactorily displayed by the microscope. In particular, the term has been employed in connexion with those germs of disease which, by reason of their extreme minuteness, are capable of passing through the very fine pores of the Chamberland porcelain filter.

There is in the use of the term "ultra-microscopic" confusion of thought—confusion between microscopic visibility and microscopic resolution.

It is not possible to contend that an element must attain certain dimensions and must subtend a certain angle before it becomes visible to the unaided eye or, as the case may be, to the eye armed with the telescope or microscope. Confining ourselves to the latter, it is indubitable that no object can be too small to figure in the microscopic image. Provided only that it forms a distinct element in the microscopic object picture it must inevitably be reproduced in the microscopic image.

It is, on the other hand, possible to contend—and the contention has been supported by weighty authority—that quite apart from the defects attaching to individual microscopes there is in each case an untransgressable limit of resolution—a limit which would make it futile to try to resolve with the microscope a ruling of less than a certain periodical interval, or, in the parallel case of the telescope, to resolve a double star whose components happen to subtend less than a particular angle.

2. Theoretical limit of resolution.

The conception of a theoretical limit to microscopic resolution was developed for the first time in the work of Helmholtz. Helmholtz takes his departure from the conception that a limit must be imposed upon microscopic resolution by the fact that the antipoint figures corresponding to neighbouring points in the object must sooner or later overlap in such a manner as finally to give, instead of separate images, a homogeneous, uniformly illuminated, surface.

Helmholtz's argument[1] may be thrown into the following form:—

(1) Every radiant point is represented in the image by a false disc, whose dimensions, are, in the case of a circular aperture, furnished by the formula[2]—

$$\text{Radius of false disc} = \frac{0{\cdot}6\,\lambda}{\text{n.a.}}$$

(2) The distance between the centres of the false discs, as measured upon the image, corresponds to the actual distance which separates the points of origin of the beams in the object multiplied by the magnifying power of the optical system.

(3) Where the false discs overlap, the light of the one will simply add itself to that of the other. By reason of such summation, resolution will become impracticable as soon as, if not before, the point is reached when the false discs overlap to such an extent that the outer edge of one disc falls upon the centre of the adjoining one.[3]

(4) The minimum distance at which objects can be disposed consistently with their separate resolution in the microscopic image will be arrived at by dividing the radius of the false discs as laid down in the ultimate image by the magnifying power of the system.

[1] I am indebted for the analysis of Helmholtz's reasoning, which I here set down in a somewhat different form to Mr. Gordon's paper *On the Helmholtz theory of the microscope* (*Journal* of the Royal Microscopical Society, 1903).

[2] *Vide* pp. 110, 111 and footnote to p. 110.

[3] As for instance in Plate IX, Fig. 3, C.

This conclusion may be thrown into mathematical form as follows :—

(*a*) Ultimate limit of resolution $= \frac{\text{radius of the false disc}}{\text{magnifying power of system.}}$

This, since the radius of the false disc corresponds in the case of a circular opening to $\frac{0{\cdot}6\,\lambda}{\text{n.a.}}$, may be written—

(*b*) Ultimate limit of resolution $= \frac{0{\cdot}6\,\lambda}{\text{n.a.} \times \text{magnifying power.}}$

Again, since, as we have seen in *Cap.* VII, *subsect.* 35, n.a. $\times$ magnifying power = N.A., we may write the formula as follows :—

(*c*) Ultimate limit of resolution $= \frac{0{\cdot}6\,\lambda}{\text{N.A.}}$

The significance of Helmholtz's conclusion emerges as soon as we see it in this shape. We recognize that microscopic resolution is, according to Helmholtz, a function of the N.A. of the objective.

Before discussing the premisses upon which this conclusion is based it will be convenient to adduce examples to illustrate the method of calculating by the aid of Helmholtz's formula, the resolving power of an objective of particular aperture.

Example 1. Required the ultimate limit of resolution for an objective possessing a N.A. of 1·4.

Taking here, as elsewhere, the value of λ as 0·0006 mm., 0·6 λ will be 0·00036, and the ultimate resolving power of the objective $\frac{0{\cdot}00036}{1{\cdot}4} = 0{\cdot}00025$ mm. (0·25 μ).

Example 2. Required the ultimate limit of resolution for an objective which possesses a N.A. of 0·3.

Ultimate limit of resolution $= \frac{0{\cdot}00036}{0{\cdot}3} = 0{\cdot}0012$ mm. (1·2 μ).

3. Question as to whether the limit of Helmholtz is the untransgressable limit of resolution.

Before attacking the problem as to whether the limit of Helmholtz represents the untransgressable limit of resolution, it will be well to appreciate the full strength of the argument which can be adduced in support of the position taken up by Abbe and others, who are in reality in this matter followers of Helmholtz.

Their position rests not alone upon the theoretical argument which has been set forth above, but also upon the fact that the limit of resolution as assigned by the formula $\frac{0{\cdot}6\,\lambda}{\text{N.A.}}$ corresponds very closely with the limits of resolving power as actually encountered when

objects are viewed by the unaided eye, or, as the case may be, by the microscope.

We have already (*Cap.* X, *subsec.* 10) dealt with the unaided eye. We may here confine ourselves to the microscope.

Working with the best objectives, e.g. with Zeiss' apochromatics of 1·4 N.A., the microscopist will generally find that he has reached the extreme limits of his resolving powers when he has succeeded in resolving rulings which lie $0{\cdot}25\mu$ apart, i.e. about 4,000 to the mm. (100,000 to the inch).

This striking agreement between the results of calculation, as given above in *Example* 1, and the result of actual experiment has very naturally procured for the Helmholtz theory wide acceptation.

The wide acceptation of the theory does not, however, absolve us from the necessity of inquiring into the cogency of Helmholtz's argument, and, in particular, into the validity of his two underlying assumptions : (*a*) that the dimensions of the antipoint, as laid down in the image, are those furnished by the formula $\frac{0{\cdot}6\,\lambda}{\text{N.A.}}$; and (*b*) that there is, in the regions of the image where adjacent antipoints overlap, a simple summation of the light. Each of these assumptions has been challenged.

Mr. Gordon has insisted that the antipoint which comes into consideration in connexion with the resolution of the image is not the theoretical antipoint whose dimensions are furnished by the formula, but the conspicuous antipoint whose dimensions are always —except as in the case of extremely brilliant illumination—much less than those of the theoretical antipoint.[1]

Lord Rayleigh has further pointed out that the independence of phase, which is a *sine quâ non* of the summation[2] of the light of overlapping antipoints, would not be obtained in the case where beams derived from adjacent radiant points follow in the microscope paths which intersect under a very acute angle.

It is thus manifest that the postulates of Helmholtz cannot be accepted as assured.

This is not all. There is gradually accumulating a body of

[1] It will be remembered in this connexion that we have already, in *Cap* IX, *Appendix* I, *Experiments* 2 and 3, seen evidence of the correctness of Gordon's contention in the fact that a system of rulings which yields when illuminated by direct sunlight an unresolved image, furnishes on the reduction of the illumination a perfectly well resolved image.

[2] A summation effect such as is here in question is in Plate IX, Fig. 3 C, represented in the regions where the lateral discs overlap the central disc.

evidence, derived from trustworthy microscopic observers, which goes to show that objects whose elements lie beyond the theoretical limit of Helmholtz have on many occasions been satisfactorily resolved by the microsope.

4. Question as to the real nature of the limit to resolution which is encountered by the microscopist in connexion with the employment of high magnifications.

If the Helmholtz theory is to be abandoned, it will be asked whether any other explanation can be furnished of the fact that both in the case of the unaided eye and when working with the best microscopic objectives, difficulties in the matter of resolution are encountered as soon as the limit of Helmholtz is arrived at.

There is not, in point of fact, any difficulty in suggesting an explanation of this circumstance. We have to realize that there are three different factors which may interfere with microscopic resolution; that each of these separately may place a limit upon resolution; and that all three of these will work in conjunction to impair resolution as soon as we advance to high magnifications.

The three impediments to resolution just referred to are; (*a*) diffusion, (*b*) conspicuous antipoint, and (*c*) obfuscation in the eye.

The last two of these are, as we have seen, directly referable to the contraction of the beam which is associated with high magnification. The first also, which is primarily dependent on defective correction, may be brought into relation with the contraction of the beam, inasmuch as further progress in the direction of the elimination of diffusion is in each case arrested as soon as conspicuous antipoint and increasing obfuscation in the eye interfere with the detection of uncorrected errors in the objective.

5. Is it possible to advance to a higher limit of resolution than that attained by the present optical arrangements?

It will be obvious to consideration that the advance to a higher limit of resolution will be conditional upon the successful elimination of the impediments to resolution enumerated in the last subsection. Further, it will be plain that these impediments will be removed only if it proves possible to open up the terminal beam.

6. On Gordon's method of opening up the beam before it is delivered to the eye by the microscope.

From the time when the disadvantages which follow from the excessive restriction of the terminal beam were first recognized,

the efforts of opticians have been directed to opening up that beam. The introduction of oil-immersion lenses, and, in particular, the progressive increase in the numerical aperture of objectives which was inspired by Professor Abbe, are to be regarded as steps towards the attainment of this end. With the attainment of what has already been attained in the direction of the opening up of the numerical aperture of the objective, and with the acceptance on the part of opticians generally of Helmholtz's theory, which seemed to proclaim all efforts in the direction of further microscopic resolution as vain, unless objectives of larger N.A. could be achieved, the development of the microscope seemed destined to come to a definite standstill.

Only recently has a new prospect been opened up. Mr. Gordon has called attention to the overlooked fact that besides the method of expanding the numerical aperture of the objective and admitting

Fig. 95.

A Apertural plane of the first objective.
B Apertural plane of the second objective.
C Apertural plane (Ramsden disc) of the eye lens.
D Ground glass screen mounted in the image plane of the first objective.

a larger beam into the microscope, there is yet another method by which the terminal beam may be opened up. The beam may, as it was given to Mr. Gordon to discern, be opened up subsequent to its admission into the microscope ; and it may be opened up to such an extent as to allow of any desired magnification being employed.

Mr. Gordon's suggestions have taken shape in his *tandem microscope*. The essential features of this microscope are as follows :—

(1) The image which is furnished by the objective and field lens is received upon a finely ground glass disc which is disposed in the image plane of the objective field lens combination.

(2) In the course of its passage through this screen the beam is opened up by refraction, so as to follow the path indicated

in the diagram by the unbroken lines, instead of the path indicated by the broken lines.

The function here subserved by the ground glass is the same as that subserved in the case of the photographic camera by the focussing screen and in the case of the projection lantern by the canvas on which the magnified image is projected.

(3) The beam thus opened out is received into a second objective, which is mounted behind the first objective and field lens.

(4) This second objective is surmounted by an ordinary eye piece, which brings to focus upon the retina the image of the object upon the stage of the microscope, in association with the image of the screen, which is disposed in the terminal focal plane of the first objective field-lens vista.

(5) From this composite image there is deleted, by a special device, that element in the composite image which is referable to the grain of the ground-glass screen.

It will be recognized that if in the case of the image furnished by the projection lantern on a canvas screen the grain of the screen can be neglected, it can in this case be neglected because the image is viewed with the unaided eye and not through a magnifying system.

(6) The device which is employed for this end is the device of setting the screen in motion in an appropriate manner. The details of the mechanism, which is employed for this purpose, are explained and illustrated in the Appendix which Mr. Gordon has very kindly supplied for this Chapter.

7. Appraisement of Mr. Gordon's achievement.

Mr. Gordon's achievement can be properly adjudicated upon only when it is clearly realized that the method he has proposed is not a method for remedying diffusion by perfecting the corrections of microscopic objectives, but a method for eliminating from the retinal image those defects which depend upon the contraction which the beams, which take origin upon the stage, undergo in the course of their passage through the microscope.

It follows that in appraising Mr. Gordon's achievement we have to take into account only two questions ; *first*, the question as to whether the ground-glass screen which he employs does, or does not, open up the transmitted beam in such a manner as to purge the retinal image from conspicuous antipoint and obfuscation ; *secondly*, the question as to whether the movement which is

imparted to the screen does or does not satisfactorily eliminate from the composite image on the retina all those elements which are derived from the grain of the screen.

On both issues the answer must be in the affirmative.

Mr. Gordon has repeatedly demonstrated, both at the Royal Microscopical Society and elsewhere, that it is possible by the aid of his apparatus to obtain, in association with magnifying powers of 10,000 diameters and over, retinal images which are absolutely free from obfuscation and conspicuous antipoint. Again, upon occasions when the mechanical appliances for imparting movement to the screen have been working satisfactorily, he has been able to show that the structure of the screen may be, all but absolutely, obliterated from the retinal image.

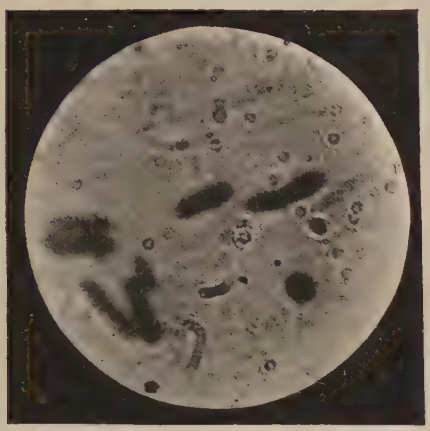
Inasmuch as the retinal images cannot be photographed, the evidence cannot be reproduced. But there can by superposing a camera upon the eye lens of the microscope be furnished here images which conform with the retinal images in every respect except only in the matter of the blemishes which are imported into the picture during the passage of the very narrow beams through the observer's eye. On Plate XVIII are photographs of one and the same group of typhoid bacilli taken by the aid of Mr. Gordon's microphotographic apparatus [1] employed in connexion with his tandem microscope.

In A, where the ground-glass screen has not yet been introduced into the microscope, and where consequently the aperture of the beam which emerges from the eye lens is reduced proportionately to the magnification, we have scattered all over the field of the image black spots surrounded with diffraction rings. These spots and diffraction rings are derived from impalpably fine particles of dust on the surface of the eye lens. They acquire the prominence they have here by reason of the fact that they lie athwart extremely narrow beams.

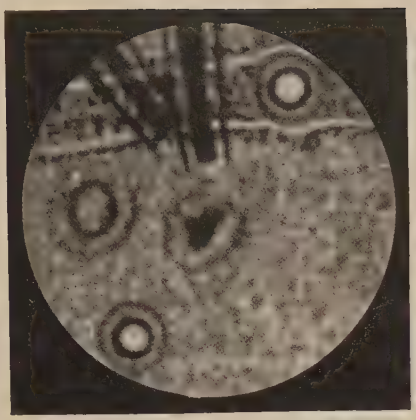
In D, where the ground-glass screen has been introduced into the barrel of the microscope, attention is immediately arrested by the fact that a magnified image of the grain of the screen is now superimposed upon the picture. To be noted also in connexion with the picture is the fact that the shadows and diffraction patterns derived from the dust particles on the eye lens have

[1] The apparatus here in question is constructed upon the principles of the eikonometer—the micrometrical scale of that apparatus being replaced by a photographic plate.

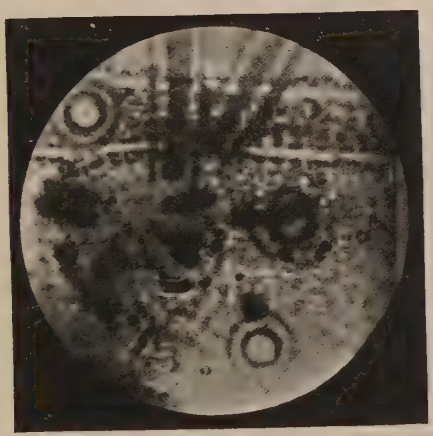
PLATE XVIII.



A

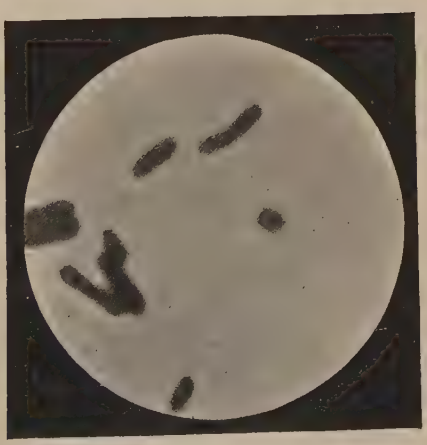


B



C

D



E

entirely disappeared. Their fading out is the result of the opening [1] out of the transmitted beam which is accomplished by the ground-glass screen.

In E the ground-glass screen has been set in motion in the barrel of the microscope, the resultant picture corresponding exactly to D except only in the respect that the grain of the ground-glass has been effaced from the image by the movement which has been imparted to the screen.

It is to be noted in conclusion that the difference between the retinal images which are obtained with very high powers, employed with and without the Gordon screen, cannot be fully gauged by comparing the photographs A and E. In the case of the image furnished by the contracted beam (A) there would be superposed upon the image, as we have seen in *Cap.* XII, *subsect.* 18, an entoptic picture such as is shown in B.[2] The difference that this would make to the retinal image can be learned on turning to C, where by composite photography the entoptic image B has been superposed upon A. In the case where, as in E, the image is produced by an expanded beam the entoptic picture would be quite inconspicuous.

The difference between C and E on Plate XVIII may thus be taken as corresponding to the difference between the retinal images obtained with and without Mr. Gordon's oscillating ground-glass screen.

8. Question as to whether Mr. Gordon's achievement opens up a prospect of an advance towards increased resolving power.

It having been made clear that Gordon's method aims at objects other than an increase of the resolving power of the lenses which we have at present at disposal, and that it achieves those objects; there still remains the question as to whether it is likely to carry us on towards the achievement of a higher limit of resolution.

In this connexion the following may be considered.—The optician who is endeavouring to learn whether his objective is properly corrected by examining under high magnification the image

[1] The opening out of the beam is attested to the observer who is dealing with the tandem microscope by the fact that the Ramsden disc of the eye lens expands as soon as the ground-glass screen is placed in position.

[2] We have seen above (*Cap.* XII, *subsect.* 18) that a projection picture of the pupillary aperture such as is shown in Plate X, Figs. 4 and 5, and Plate XVIII, B, is obtained whenever the microscope delivers a homocentric system of very narrow beams to the eye.

which is furnished by his microscope is in reality in much the same plight as a man condemned, in selecting spectacles for himself, to view his test objects through a pin point aperture placed in front of his pupil. Clearly the glasses that would be chosen by a man working under these disadvantages would much less adequately correct his eye than those he would have chosen if he had been working with unrestricted pupil.

Basing ourselves upon this, we may reasonably anticipate that when the optician comes to scrutinize his highly magnified microscopic images under the more advantageous conditions which can be realized by the aid of Mr. Gordon's apparatus, he will find it possible, by still further refining upon the corrections and adjustments of his lenses, to supply an objective which will, when employed in connexion with Mr. Gordon's microscope, achieve a higher limit of resolution than has yet been attained.

Appendix to Chapter XVI.

The subjoined figure below illustrates the "tandem" microscope, provided with an oscillating screen, which has been referred to in *subsect.* 6 and 7, *supra.*

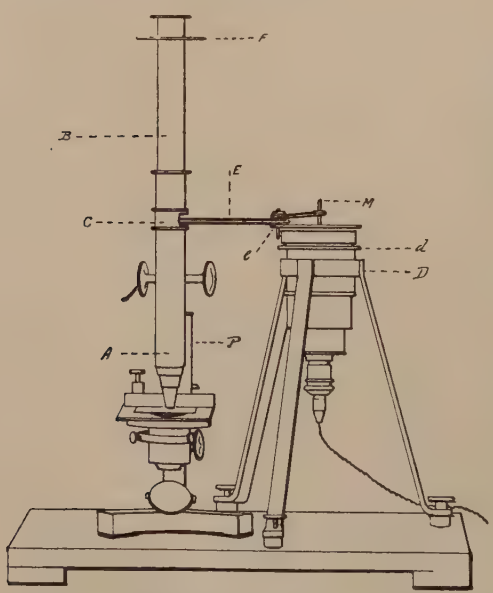


Fig. 96.

The microscope proper, marked *A* in the diagram, is of the ordinary construction, provided, however, with a clamp, not shown in the diagram, which grips the rack and, resting upon the top of the pillar *P*, supports the weight of the second microscope, which otherwise would tend to turn the pinion of the coarse adjustment and disturb the focus of the instrument. This is rendered necessary by the weight of the added parts.

These added parts consist of the tube and objective and ocular of a second microscope *B*, which are mounted in place of the eye lens of the principal microscope *A*. Thus, the image formed in the

image plane of the principal microscope is viewed in this arrangement not through a simple eye lens, but by means of the compound microscope *B*, and therefore under greatly increased magnification. The microscope *B* fits by means of a screw, or other convenient means of attachment, over the connecting piece *C*.

This connecting piece *C* takes the place of the draw-tube in the ordinary arrangement of the microscope, and is indeed a draw-tube of special construction. That is to say, it carries the field lens of the principal microscope in the usual position, and has a gap, shown on the right-hand side of the diagram, through which the oscillating screen and the end of the arm by which it is carried can be admitted to the tube of the instrument at the level of the image plane of the principal instrument. The eye lens, removed from the ocular of the principal microscope to make way for the added instrument, is mounted in the ocular of this secondary microscope *B*. Thus the total magnifying power is increased by the magnifying power of the added combination, that is to say, of the combined objective and field lens of the second microscope. This may be taken to amount for a short tube length, such as is suitable for this instrument, to × 6, for, say, an added object glass of $\frac{1}{2}$ in. focal length. Thus an instrument which with a mere eye lens would give a magnification to the eye of × 1,000 diameters, may in this way be increased to a power of 6,000 diameters. The upper microscope is focussed by means of the focussing ring *F*.

It will be understood that no ordinary microscope will stand a magnifying power approaching to 6,000 diameters. In fact, microscopes apart, no human eye will stand such power under ordinary conditions of presentation. The structure of the crystalline lens and floating and other opaque particles in the eye become so very manifest, that the image so formed is wholly unserviceable, and technically known as "rotten." To get over the difficulty so arising, a diaphanous screen is introduced into the image plane within the connecting member *C*, and receives the image formed by the principal objective. This screen, which may be of finely ground glass, breaks up the optical system and spreads the transmitted beams of light over a wider angle than the angle of the incident beams. Thus the optical system of the upper instrument starts from a new datum, the angle of the light supplied to it being quite independent of the magnification effected by the principal instrument, and determined only by the dispersive power of the grain of the screen and the light-gathering power of the second

objective. Thus the final image exhibited by this piece of apparatus is just as easily visible and as free from adventitious features as an image of lower magnification.

The diaphanous screen which effects this transformation is carried by the tripod *D*, provided with padded feet and elastic supporting connexions for the purpose of cutting off vibrations from the stand. Within the supporting ring of this tripod stand are various interior rings for giving the necessary movements in height and azimuth, to enable the arm *E*, which carries the screen, to be adjusted in position. This arm is movable in rotation about the axis of the supporting ring of the tripod stand, and has an elbow joint at *e*, so that its free end can be introduced into the microscope tube (as shown in Fig. 96), or swing clear of it at the observer's option. The ring *d* operates a screw for finely adjusting the level of the arm, and, therefore, of the screen when in use.

In order to obliterate the grain of the screen, it is kept in motion, and for this purpose a motor *M* is carried in the axis of rotation of the screen-carrying arm. Elastic bands transmit the power from the driving pulley of the motor to a driven pulley carried at the free end of the arm. The detail of this mechanism is shown in Fig. 97 below.

Here *P* is the driven pulley, mounted on a perforated stud *S*. Through the lumen of this stud the image-forming beam of the principal microscope passes, and on its flat top the ground-glass screen rests. The pulley *P* carries, as shown, an eccentric ring *R*, made of a diameter to receive the diaphanous screen in a loose embrace. As the pulley revolves this ring will therefore pull the screen round with it, but without lifting it from the surface of the stud. The friction of this latter will therefore cause the screen to revolve backwards within the ring, and the whole result is that no point upon the screen moves in a closed path, and therefore the whole becomes invisible at a very moderate speed of rotation. With a half-inch objective it is found that from seven to ten revolutions a second give a very satisfactory picture to the eye, and that very much slower speeds—depending, of course, upon the intensity of the illumination—and, by consequence, upon the time of exposure—suffice for photo-micrography.

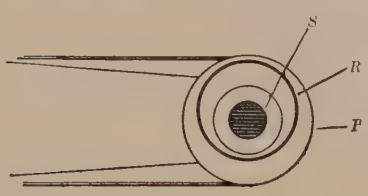


Fig. 97

INDEX

Abbe theory of microscopic vision—124–129; *see* also *Abbe's diffraction plate*.

Abbe's diffraction plate—112; experiments with, —— 112–115, 125–126, 127–128.

Aberration—*See* under *Spherical aberration* and *chromatic aberration*.

Achromatic objectives—99.

Angular aperture (employed throughout in the sense of *angular sine aperture*, and not in the sense of *angular arc aperture*)—73; simple, as distinguished from numerical, aperture, 73–74.

Anilin dyes—classification into "acid" and "basic" dyes, 34; solubilities of —— 34, 35, 36. *See* also under *Staining*.

Antipoint—definition of —— 106; —— of a luminous point seen through a restricted circular opening, 106–107; method of bringing this —— into view, 107; —— of a luminous point viewed through a slit aperture, 107; method of bringing this —— into view, 107; disposition of this —— with reference to the slit aperture, 108, Exp. 2; —— pattern of a file of points as seen through a slit aperture, 108; as seen through a restricted circular aperture, 108; method of bringing this into view, 108, Exp.; —— of a luminous surface, 109; distinction between "theoretical" and "conspicuous ——" 109; relation in which latter stands to the brilliancy of the source of light, 109; law which governs dimensions of the theoretical —— as furnished by a simple aperture, 109–111; practical importance of —— in connexion with the microscopic image, 111; —— of the eye; effect of reducing numerical aperture of eye on dimensions of —— 139.

Apertural plane.—*See Point vista* and *Surface vista*, optical anatomy of.—Progressive reduction in dimensions of —— goes hand in hand with progressive magnification, 89, 93–94, 138.

Aperture—role of the —— in connexion with formation of image, 51; relation between dimensions of —— and brilliancy of image, 76; relation between dimensions of —— and sensitiveness to alteration of focussing adjustment and resolution in depth, 76; relation between dimensions of —— and liability of image to occultation by intrusive objects, 77, 83–85; relation between —— and clear definition of image in case of interpenetrating vistas, 78, 82–83.

Apochromatic objectives—99.

Barrett, Prof. W. F., Papers on entoptic vision referred to—142.

Basophil tissue elements—34.

Blood film—preparation of dried ——s, 22; dark outline picture of —— 22, Exp. 1; bright outline picture of a —— 23, Exp. 2; effacement of outline picture, 23, Exp. 3; picture in relief of —— 23, Exp. 4; colour picture of —— 24–25; picture in colour and relief of a —— 25, Exp. 2.

Blue mottling of red blood corpuscles, suggested explanation of — 45.

Bright outline picture (*dark ground illumination*)—definition of and familiar examples of —— 3–4; experiments in production of —— in connexion with macroscopic objects, 12; in case of objects placed on stage of microscope, 20; defects of, considered as a stage picture, 23, Exp. 2, and 40;

adjustments of condenser required for development of —— in connexion with narrow angled objective, 173; conditions which must be realized for achievement of —— in association with wide-angled objectives, 211–217. *See* also under *Dark ground illumination.*

Catena of vistas—definition of —— 85; various configurations of —— 86; law governing inversion in —— 87; methods of measuring scale of image obtained on terminal plane of —— 87-88; determination of scale of image by dividing numerical aperture of opening angle by numerical aperture of closing angle, and examples, 88–89; progressive magnification in —— associated with progressive constriction of the transmitted beam, 89, 93–94. *See* also *Concatenation of vistas.*

Caustic—95.

Centring cap—180.

Centring—purport and method of —— the condenser, 179; —— of the objective, 194–195; importance of —— in connexion with the achievement of a critical image, 222–223.

Chromatic aberration—98–99; method of correcting —— 99.

Chromophor—definition of —— 34; classification of ——s into acid and basic, 34.

Clearing of microscopic sections—definition of —— 31; illustrative experiments, 29–30, Exps. 2–7; method of clearing where interstices are filled (*a*) with air, (*b*) with water, 31–32; employment of alcohol, oil of cloves and Canada balsam for —— 31–32; popular error as to clearing agents, 32.

Colour picture (*synonym*, pure colour picture)—definition of —— 3; experiments on production of pure —— 23, Exp. 3; fallacies which may be associated with—*see* under *Fallacies*; —— obtained where object is mounted in a medium of equal refractive index and where objects are illuminated at one and the same time by oblique and axial rays, 18, Exps. 3–4, 23, Exp. 3; 24, Exp. 1; —— is only form of stage picture which can be achieved where the elements in a microscopic preparation are piled one upon another, 30–31; method of obtaining colour picture by staining, Chap. IV *passim*; —— can be achieved only where dyes are available which have with respect to the microscopic objects which are to be depicted elective chemical affinities, 40; disadvantages and imperfections which are associated with —— 44–45; the opening up of the numerical aperture of the condenser which is favourable to achievement of this form of stage picture is favourable also to achievement of a critical image of the stage picture, Chap. XV.

Combined outline and colour picture—definition of —— 3; familiar examples of —— 4; disadvantages of —— in case where measurements are to be made, 6, 21, Exp. 4; —— obtained where a coloured object which is mounted in a medium of less refractive index is illuminated by luminous points which radiate light under a very small angle, 18, Exp. 3. This form of stage picture to be avoided, 4–6.

Compensation oculars—100.

Concatenation of vistas—*See* under *Catena of vistas.*

Condenser (Substage)—method of focussing —— 173–179; method of centring —— 179, 180; method of immersing —— 180, 181; focal length of —— 181; method of measuring focal length of —— 181; numerical aperture of —— 181, 182; limitation of numerical aperture of —— where employed as a dry system, 183; method of measuring numerical aperture of —— 182–183; method of obtaining from the Abbe condenser a wider beam that corresponds to numerical aperture of —— 183–184; corrections of —— 185–186; method of testing corrections of —— 187; principles in accordance with which the working aperture of the condenser ought to be regulated, 223–224.

Condenser vista—*See Vista of the substage condenser.*

Contrast in the microscopic image—lost by overlap of antipoints, 111.
Correction collar—*See* under *Objective.*
Corrections for spherical aberration—97, 98; for chromatic aberration, 99; —— in achromatic objectives, 99; —— in apochromatic objectives, 99.
Coverglass—method of correcting for different thicknesses of — 201, 226; effect of —— in distorting the opening limb of the objective vista, 191; employment of —— obligatory in connexion with dry objectives, unnecessary in connexion with oil immersion objectives, 193.
Critical image—adjustments for achievement of Cap. XV, regulation of source of illumination, 219; centring of condenser of —— 222, 223; regulation of aperture of condenser, 223, 224; choice of objective, 224; choice of ocular, 227.
Cylinders of coloured and uncoloured glass for optical experiments—method of making —— 11.

Dark ground illumination—obtainable both by the ordinary arrangement ("the arrangement by which the preparation is illuminated by hollow and imaged by solid beams") and by the "arrangement by which the preparation is illuminated by solid and imaged by hollow beams," 211–213; employment of the former arrangement in connexion with low-power narrow-angled objectives, 20, Exp. 2; employment of the latter arrangement in connexion with narrow-angled low-power objectives, 214; employment of the former arrangement in connexion with wide-angled high-power objectives, 214, 215; method of Siedentopf and Zsigmondi, a variant of this arrangement, 215, 216; claim that this method reveals elements of ultra-microscopic minuteness criticised, 229; employment of the "arrangement by which the preparation is illuminated by solid and imaged by hollow beams" in connexion with wide angled high power objectives, 216, 217. *See* also *Bright outline picture.*
Dark outline picture—definition and familiar examples of —— 3, 4; experiments on production of —— in case of macroscopic objects, 12; in case of microscopic objects placed on stage of microscope, 20; fallacies in connexion with —— *see* under *Fallacies*; defects of —— considered as a stage picture, 40–44; adjustments of condenser required for development of —— 172; the restriction of the numerical aperture of the condenser which is essential to development of —— is inconsistent with development of a critical image in association with high magnification, Cap. XV.
Dark points produced in crenated red blood corpuscles by deflection of light, possibility of confusing these with granular pigment, 44.
Definition—meaning of —— as employed in connexion with the microscopic image, 54 *note.*
Definitive focal plane—absence of —— in case of dark outline picture, 41.
Derivative picture—definition of —— 7; possibility of confusion with *fundamental picture*, 41. *See* also under *Fallacy of misprision of focus.*
Diaphragms—substage diaphragms—iris diaphragm, 169; retro-objective diaphragms of Abbe employed in connexion with the study of diffraction, 112–115, 124–129; Abbe's retro-objective diaphragm with three slit openings functions as diffraction grating, 123. *See* also under *Stops.*
Diatoms—use of as test objects for proving of objective, 203.
Differential staining—37, 38.
Differentially lighted picture—12, Exp. 4; manner in which the picture is produced, 17; 21, Exp. 5. *See* also under *Rheinberg.*
Diffracted beams—106.
Diffraction, theory of—*See* under *Undulation theory*; —— in aperture of lens, Cap. IX; effect of —— on the resolution of the image, 112; experiments showing effect of —— on resolution, 112–116; —— as producing spurious detail,

116–120 (*see* also under *Fallacies referable to diffraction*); —— occurring in object plane, Cap. IX., Appendix I.
Diffraction grating—advantages of a —— over a simple aperture for study of outlying elements of antipoint, 116; experiments with —— 117; dimensions of antipoint furnished by —— 118, 119; simple form of —— ruled with 400 lines to the inch (sixteen lines to the millimetre) supplied with this book; more permanent form of same —— obtainable from Messrs. Sanger Shepherd, Gray's Inn Place, London, W.C.; method of measuring magnifying power by aid of a —— 129–131.
Diffraction pattern—definition, 106. *See* also *Antipoint.*
Diffraction patterns—produced by dust on eye lens, 228, 236.
Diffraktionsplatte (Abbe)—*See Abbe diffraction plate.*
Dioptric beam—definition of —— 106.
Doubling of rulings—*See* under *Fallacies referable to diffraction.*
Draw tube and barrel of microscope—length of —— in Standard English pattern, 205; in Continental pattern, width of —— 205, 206.
Dust on surface of coloured microscopic specimen may give rise to fallacy under influence of subjective colour contrast, 45.

Eikonometer—description of author's —— 145, 146; method of employing the —— for measuring the magnifying power of the simple microscope, 146, 147; procedure for measuring by the aid of the —— the magnifying power of the compound microscope, 163, 164.
Elective affinity of stains for particular tissue elements—35.
Emery paper—use of —— in connexion with preparation of dried blood films, 22.
Entoptic picture and conditions under which it is obtained in connexion with unaided eye—140–142; superimposition of —— upon the microscopic picture, 167, 237; critical image obtained only when by opening up the beam which is delivered to the eye, provision is made for suppressing the entoptic picture, 222–225; limits are imposed upon microscopic vision by the increasing prominence of —— which is associated with high magnification, 94, 233. Mr. Gordon's device for opening up the transmitted beam achieves effacement of —— 235–237.
Equatorial plane—*See Apertural plane.*
Eye—*See Unaided eye.*
Eyelashes—obstructive interference by —— 137, 138. *See* also 167, 237.
Eye lens and eye vista—157–159.
Eye vista—*See Eye lens and eye vista.*

Fallacy of misprision of focus—definition of —— 7; examples of —— 7, 8, 42, 43; —— in connexion with diatoms used as test objects for proving objective, 203–204.
Fallacy of subjective colour contrast—definition of —— 8; conditions under which —— may occur, 9; examples of —— 12, Exp. 6, 21, Exp. 6, 30, Exp. 4, 45 (*a*) and (*b*).
Fallacy of mistaking dark points due to deflection of light for pigment, 43, 44.
Fallacies referable to diffraction—widening out of rulings, 112–115; introduction of *spurious detail* into the image in the form of *intercostal spots*, 116, and *intercostal lines*, leading to doubling and trebling of rulings, 120–123.
False disk—definition of —— 106.
Field of view—measurement of —— 81; restriction of —— by pupillary border of the iris when light enters the eye in the form of a homocentric beam, 140–142; restriction of —— in case of simple microscope by pupillary border of the iris, 148, 149; restriction of —— in case of compound microscope by pupillary border of iris where the pupil is disposed behind the Ramsden disk of the eye lens, 158.
Focussing—importance of the focussing adjustment, 194.
Fundamental picture—definition, 7. *See* under *Fallacy of misprision of focus.*

Gordon, (J. W.)—suggestion for reducing dimensions of light source for achievement of critical image, 221; device for opening up the beam when in transit through the microscope, 233–235; question as to whether this device opens up a prospect of advance towards increased resolving power, 237, 238; detailed description of his tandem microscope, 238–240; suggestion for employment of a retro-ocular central spot stop for achievement of dark ground illuminations, 217; explanation of doubling of rulings by the treble slit diaphragm of Abbe apparatus, 123; photomicrographic apparatus, 233 *note*.

Homocentric beam—definition of —— 140; conditions under which obtained, 140, 149, 167 (*see* also *Entoptic picture* and *Projection picture of the pupillary aperture*).

Image—essential element in —— formation is isolation of light derived from any object from light derived from surrounding objects, 51. *See Simple aperture image* and *Lens image*.

Immersion objectives—advantages of —— 192–193.

Immersion of the condenser—*See* under *Condenser*.

Immersion medium—effect of an immersion medium of high refractive index, on the focal length of the lens, 74; allowance is to be made for this artificial lengthening out of the focal length in calculating numerical aperture of lens, 74–75; *See* also under *Immersion objectives*, *Immersion of the condenser* and under *Coverglass*.

Intercostal lines—115; development of ——— in such a manner as to produce doubling or trebling in a periodical ruling, 120–123; employment of —— for purposes of measuring the magnifying power of a lens system, 129–131.

Intercostal spots—115–116; experiments on production of —— 116.

Interpenetrating vistas—definition of —— 78; influence of aperture upon the images formed in interpenetrating point vistas, 78; and interpenetrating surface vistas, 82–85.

Intrusive vistas—vide *Interpenetrating vistas*.

Inversion—(in the sense of change from dark to bright and vice versâ, taking place on altering the focus) 8, 43, Exp. 3, 4 and 5. *See* also *Fallacy of misprision of focus*.

Inversion—(in the sense of the representation of an upright object by an upside down image) —— in simple aperture image, 52, Exp. 1; —— in lens image, 65; —— in a catena of vistas, 87; —— in the unaided eye, 133; psychological factors in connexion with this last, 133; —— in simple microscope, 143; —— in compound microscope, 151–154.

Invisible pathogenetic micro-organisms —explanation of their invisibility, 47–48. *See* also under *Ultra-microscopic elements*.

Iris diaphragm—169. *See* also under *Stops*; arrangement of —— for obtaining oblique illumination from, 171.

Jenner's stain—37.

Koch, R.—classification of microscopic pictures, 3 *note*.

Lagrange disk—*See Surface vista* (*optical anatomy of*).

Leishman's stain, 24.

Lens image—effect of lens on course of beam, 63; effect of lens on configuration of beam, 64; resemblance between simple aperture and lens images in the matter of magnification achieved, 66–67; difference between simple aperture image and lens image in the matter of resolution in plan, 67, 68; in the matter of resolution in depth, 68–69; imaging of elements concealed from a direct view a characteristic feature in the —— 70.

Light source, adjustment of—*See* under *Critical image*.

Limitations of microscopic achievement—discussion of comparative importance of the limitations which have reference to

development of stage picture and limitations which have reference to development of a magnified image of the stage picture, 46–48.

Limits of microscopic resolution—Helmholtz's theoretical limit of resolution, 230–231; criticisms of by Gordon and Lord Rayleigh, 232; Impediments to microscopic resolution (*a*) diffusion, (*b*) conspicuous antipoint, (*c*) obfuscation, 233. Gordon's device for opening up the beam when in transit through the microscope abolishes "conspicuous antipoint" and obfuscation and may in this manner pave the way to a further reduction of diffusion.

Limits of microscopic vision—*See* under *Ultra-microscopic elements.*

Magnification—obtained by a vacant aperature is the same as that obtained by a lens armed aperture, 66; conditions which determine the scale of simple aperture image, 52–54; methods of arriving at the magnifying power of any vista, 80–81; methods of measuring —— obtained with the simple microscope, 144–145; for measuring —— obtained in objective vista of the mcroscope, 156; for measuring —— obtained in eye lens vista, 158, 159.

Magnifying power of objective—*See* under *Objective.*

Magnifying power of ocular—*See* under *Ocular.*

Magnifying vista—79; optical anatomy of —— 80; formulae for arriving at the scale of the image, etc., 81; application of these formulae, 81.

Microscope—optical anatomy of —— 157, 158. *See* also *Vista of substage condenser, Vista of objective, Vista of eye lens.*

Microscopic slides—effect of the —— in distorting the closing limb of the condenser vista, 190; and in disturbing corrections of any achromatic condenser, 186.

Minifying vista—79; optical anatomy of —— 80.

Misprision of focus—*See* under *Fallacy of misprision of focus.*

Muscae volitantes—in connexion with unaided eye, 137, Exp. 6. *See* also 167, 233, 237.

N.A.—explanation of this symbol, 74. *See* also under *Numerical aperture.*

n.a.—explanation of this symbol, 74. *See* also under *Numerical aperture.*

Nelson—suggestion for avoidance of difficulties associated with employment of an extended source of illumination, 220; criticism of this suggestion, 221.

Normal magnification (Helmholtz)—definition of —— 225.

Numerical aperture—purpose served by this expression, 73; definition and explanation of —— 74, 75; N.A. and n.a., 74; method of calculating, 75.

Obfuscation—definition of —— 134; experiments on effect of —— in unaided eye, 136, 137.

Object picture—definition of —— 3; classification of —— into dark outline pictures, bright outline pictures, colour pictures, outline and colour pictures, and pictures in relief, 3; advantages and disadvantages of different types of —— (*a*) from point of view of disclosure of objects which subtend only a small angle at the retina, 5; (*b*) from the point of view of the disclosure of the configuration of the object in plan and relief, 5–6; (*c*) from point of view of their greater or less liability to be vitiated in fallacy —— 7–9. *See* also under *Stage picture* and under different types of object picture.

Objective—function of —— 192; classification of ——s into dry and immersion, advantages of immersion ——s, 193; method of employing immersions ——s, 193; centring of ——s, 194; focal length and magnifying power of ——s, 195–196; methods of expressing the magnifying power of ——s, 195; method of measuring magnifying power of ——s, 196; method of measuring apertural plane of —— (*see* under *Measurement of N.A.*); advantages of large N.A., 197,

225, 234; measurement of N.A., 198, 199; corrections of —— 200–201; correction collar, 201; preliminary considerations in connexion with the testing of the accuracy of figuring and of corrections of —— 201–202; method of testing ——s, 203; choice of suitable test objects, 203–204; N.A. of —— as determining in certain cases the type of picture which is brought into view, 209–211.

Objective changers of Zeiss—205.

Obstructing elements—effect exerted by these in case of (*a*) simple aperture image, 59, 70; (*b*) lens image generally —— 70; in wide or narrow point vistas, 77; in case of surface vistas, 82–84; method of detecting of —— in apertural plane of objective, 93; —— in microscopic preparations, 29–31; —— in eye, 136, 137; —— in eye lens of ocular, 227, 228; —— in tandem microscope, 236. *See* also *Limitations of microscopic resolution.*

Ocular—Huyghenian —— 206; Ramsden —— 206; measurement of magnifying power of —— 206–207; choice of —— in connexion with development of critical image, 227; contraction of beam brought about by magnification effected by ocular —— 227; effect of dust on —— when very high magnifications are employed, 236.

Oil of cloves—function of —— in connexion with the mounting of microscopic sections, 32. *See* also under *Clearing.*

Opacity—of an object consisting of translucent elements is the result of the deflection of light by refraction and reflection, 27–28; illustrative experiments with macroscopic objects, 27–28; familiar examples of dependence of —— upon refraction and upon reflection of light from surfaces of transparent objects, 28–29; experiments with microscopic objects, 29–30.

Outline picture—(*synonym*, refraction and reflection picture) definition, 3; classification into *dark outline pictures* and *bright outline* pictures, 3; familiar examples of —— 4; cannot be obtained in a case where object is mounted in a medium of similar refractive index, 25, 26, Exp. 1 and 3; —— cannot be obtained in a case where objects piled one on another, 30; disadvantages, imperfections and fallacies which attach to the microscopic —— 40–44; —— fails to disclose objects that subtend only a very small angle on the retina, 5; absence of definitive focal plane, 41; misprision of focus, 41. *See* also under *dark outline* picture, *bright outline* picture and *outlines.*

Outlines—experiments of production and effacement of dark and bright —— (*a*) in case of macroscopic objects, 12; (*b*) in case of objects placed on the stage of the microscope, 20; (*c*) in case of blood films, 22; explanation of manner in which dark —— are produced, 14; explanation of the manner in which bright —— are produced, 15; explanation of manner in which —— are suppressed, 17; suppression of —— essential to transparency of any preparation where objects are piled one upon another, 30–31; disadvantage of the intrusion of —— into a colour picture, 6; also *see* under *combined colour and outline picture*; adjustments of the condenser which are required for the production of dark —— 171; for production of bright —— 173, 208–217.

Oxalate of calcium crystal—interpretation of the envelope-shape image obtained.

Oxyphil tissue elements—34.

Parti-coloured stops—19; method of making —— 20; —— suggested by Rheinberg for the illumination of objects by differentially coloured light, 19 *note.* *See Differentially lighted picture.*

Phantom picture—definition of —— 7. *See Derivative picture* and under *Fallacy of misprision of focus.*

Picture in relief—definition of —— 4; familiar examples of —— 4; disadvantages from point of view

of non-disclosure of internal structure of —— 6; 23, Exp. 4; method of lighting required, for development of ——— 23, Exp. 4, 25, Exp. 2, 173; incompatibility of this form of illumination with achievement of a critical image, Chap. XV. *See* also *Pictures in colour and relief.*

Pictures in colour and relief—4, 25, Exp. 2.

Plane of origin. *See Surface vista*, optical anatomy of.

Point vista—definition of —— 71; optical anatomy of —— 71, 72; relation between aperture of —— and brilliancy, resolution in depth, and imaging of elements which are masked from direct view, 76, 77.

Pole of origin—*See Point vista*, optical anatomy of.

Pupil (of lens)—*See Surface vista*, optical anatomy of; —— of entrance.

Pupillary aperture of eye—projection-picture of —— 140; eyelashes, corneal opacities, etc., silhouetted in projection-picture of —— 140.

Pupillary margin of the iris—delimitation of field of view by ——. *See* under *Field of view*

Pupillary movements—method of bringing one's own —— into view, 140.

Ramsden disk—*See Surface vista*, optical anatomy of; method of measuring —— 92; —— of eye lens and eye vista, 157–158, 167.

Refractive index of enveloping medium, —effect of —— upon development and suppression of outlines, 17–18, 25–26; effect of —— upon breadth of outline, 12, Exp. 1, 20, Exp. 1; —— upon transparency of microscopic preparations, 29, 30.

Refraction and reflection picture—*See Outline picture.*

Resolution—definition of the term as employed in connexion with the microscopic image, 54 *note*; —— *in plan* distinguished from —— *in depth*, 58; —— dependent upon concentration of the system of rays which proceeds from a radiant point in the object upon a point in the image, 68; —— affected by lens-aperture, 76, 78; —— interfered with by spherical and chromatic aberration, 95; —— interfered with by diffraction, 101, 111–115.

Resolution in depth—not obtained in the simple aperture image, 58, 59; —— obtained in lens image, 68, 69; —— runs parallel with sensitiveness of image to alterations of the focussing adjustment, 77; relation between —— and the aperture of the lens, 77; —— in unaided eye in case of mon-ocular vision determined by the dimensions of the pupil, 136, 137; —— in simple microscope determined by working N.A. of the lens, 148; —— in microscopic image determined by N.A. of objective, 196, 197; difficulties arising in connexion with —— when diatoms are employed as test objects, 203.

Resolution in plan—*See* under *resolution.*

Retro-ocular stop—*See Stops.*

Retro-objective diaphragms—*See Diaphragms.*

Retro-objective stops—*See Stops.*

Rheinberg's parti-coloured substage stops, 19 *note*. *See* also *differentially lighted picture.*

Sanger-Shepherd—diffraction gratings made by —— 116 *note.*

Siedentopf and Zsigmondi's method of dark ground illumination—215; claim that their method reveals elements of "ultra-microscopic" minuteness, 215–229.

Simple aperture image—conditions which determine scale, brilliancy, definition and resolution in plan in the case of the —— 54–58; resolution in depth not obtained in —— 59; obstructive interference in —— 59; dilemma of the —— 60.

Slide and coverglass and media—interposed between condenser and objective, 188; influence of —— upon numerical aperture of transmitted beam, 188–190; distortion of transmitted beam effected by —— where immersion is not resorted to, 190–192.

Spectacles—projection picture of pupillary aperture obtained from raindrops on —— 142, *note* 2.

Spherules of coloured and uncoloured glass for optical experiments—method of making —— 11.
Spherical aberration—95–97; method of correcting —— 97, 98.
Spurious detail—115; —— obtained in a characteristic manner by superimposition of outlying elements of two or more antipoint systems. *See* under *Intercostal spots* and *intercostal lines.*
Stage picture—definition, 3. *See* also *Object picture.*
Staining—principles of —— Cap. IV., 33–38.
Stereoscopic effects—4. *See* also under *Picture in relief.*
Subjective colour contrast—conditions which are favourable to production of —— 8, 9, 12, Exp. 6; 21, Exp. 6; 30, Exp. 4. *See* also under *Fallacies.*
Stops—*substage* ——, central spot —— 171; method of extemporizing —— 20; —— for oblique illumination, 173, 23, Exp. 4; coloured —— 20; *retro-objective* —— (for dark ground illumination), 217; (for the study of diffraction in the aperture of the objective) *see* under *Diaphragms*; *retro-ocular* central spot —— 217.
Structure picture—3 *note.*
Struktur-bild (Koch)—*See Structure picture.*
Subjective colour contrast—definition of —— 8. *See* under *Fallacy of subjective colour contrast.*
Substage condenser—*See Condenser.*
Substage diaphragm—*See* under *Diaphragms* and *stops.*
Suppression of outlines—*See* under *Outlines*; —— essential to achievement of transparency in microscopic specimens, 29–31.
Surface vista—definition of —— 71; optical anatomy of —— 79–80; influence exerted by aperture of —— upon resolution in plan and in depth and upon the imaging of elements which are masked from direct view, 82–84; conception of a vista as a sequence of radiant planes, 84–85.

Tears silhouetted upon retina when a homo-centric beam falls into the eye, 142; when a very narrow beam is furnished to the eye by the microscope, 237.
Terminal plane—*See Surface vista*, optical anatomy of.
Test objects for proving general accuracy of the corrections in the case of an objective—204; special —— for proving the accuracy of the chromatic corrections, 204; —— for testing the flatness of field, 204; criticism of employment of diatoms as —— 203.
Transparency or opacity of microscopic preparations—experiments with glass spherules piled one upon the other, 27–28; —— with microscopic sections and strips of filter paper, 29.
Trebling of rulings—*See* under *Fallacies referable to diffraction.*
Tubercle bacillus—discovery and identification of —— by staining, 47; preparation of the —— mixed with other bacteria and differentially stained, provides a satisfactory test object for proving the chromatic corrections of objectives, 204.

Ultra-microscopic—criticism of the term —— 229; in reality no object can be too small to be seen, 229; claim made by Siedentopf and Zsigmondi that their method of dark ground illumination furnishes a method for revealing particles of —— minuteness, 215 *note.*
Unaided eye—scale of image in —— 133; inversion of image in —— 133; psychological factors involved in estimation of —— 133; resolving power of —— 134; factors which influence quality of image furnished by —— 134, 135; numerical aperture of —— 135; effect of contraction of numerical aperture of —— 136–138; Helmholtz's limit of resolution applied to —— 139, 140.
Undulation theory of light—102–105.

Vista—conception of a —— 71. *See* also under *Point vistas* and *surface vistas, interpenetrating vistas, intrusive vistas, catena of vistas.*

Vista of apertural planes of microscope—configuration and optical anatomy of —— 161.
Vista of eye-lens and eye—configuration and optical anatomy of —— 157–159.
Vista of objective and field lens—configuration and optical anatomy of —— 154–157.
Vista of substage condenser—configuration and optical anatomy of —— 159.

Butler & Tanner, The Selwood Printing Works, Frome, and London.